When combined with the clinical hold response text beginning on page 57, this represents the current approved CMC section of this IND. It can be used as a template for other Zr-89 labeled antibodies.

Information Amendment to ^{89}Zr-panitumumab IND Amended Item 7 (Chemistry, Manufacturing, and Control Data)

ITEM 7. CHEMISTRY, MANUFACTURING, AND CONTROL DATA

7.1 Product Summary

7.2 Controls for Components and Raw Materials

7.3 Reference Standards

7.4 Manufacturing of Drug Substance

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7.12 Information to Support the Stability of the Drug Substance

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7.1 Product Summary

[89]Zr-panitumumab

[89]Zr labeled desferrioxamine (DFO) conjugated panitumumab is a radiolabeled monoclonal antibody. The schematic structure for [89]Zr-panitumumab is provided in Figure 7-1, below. The specifications for the Final Product are provided in Table 7-1.

7.1.1 Schematic Structure

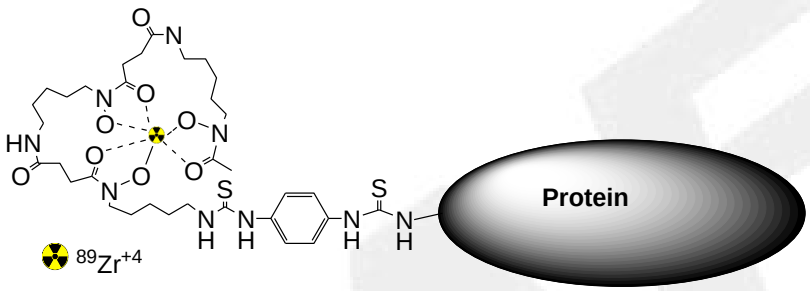


Figure 7-1. Schematic Structure for [89]Zr-DFO-Panitumumab

7.1.2 Specifications for [89]Zr-panitumumab Final Product

Table 7-1. [89]Zr-panitumumab Final Product Specifications

Test	Specification
Chemical Purity (particulates):	Clear and Colorless
pH:	6-8
Gentistic Acid:	≤5 mg per dose
Chemical Purity (HPLC):	protein <1 mg per injected dose
Radiochemical Purity (HPLC):	>95%
Radiochemical Purity (TLC):	R_f <0.5, Purity >90%
Radionuclidic Purity:	Measured half-life 78 hours
Residual Solvent Levels:	none
Specific Activity:	≥1.5 mCi/mg EOS
Bacterial Endotoxin Levels:	<175 EU per dose
Sterility:	no growth observed in 14 days; must also pass filter integrity test prior to injection

7.1.3 ^{89}Zr-panitumumab Drug Product Components and Composition

Table 7-2, below, provides the final product components and composition for ^{89}Zr-panitumumab.

Table 7-2. ^{89}Zr-panitumumab Final Product Components

Components	Amount in Injectate
^{89}Zr-panitumumab	<1.5 mCi
DFO-Protein (panitumumab)	<1 mg
Gentistic Acid	<5 mg
Saline for Injection	Remainder

The name of the drug is [^{89}Zr]-DFO-panitumumab) or ^{89}Zr-panitumumab. ^{89}Zr-panitumumab is the only active ingredient; it is injected in a solution of <10 mL of 0.9% saline (USP). The drug product solution is stored at 2-8°C temperature in a gray butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of 48 hours. The injectable dose of ^{89}Zr-panitumumab for the initial clinical study will be <1.5 mCi with a specific activity ≥1.5 mCi/mg (≥220 mCi/µmol) at the end of synthesis. The amount of injected drug is <1 mg (<7.0 nmol) of protein; i.e., panitumumab. ^{89}Zr-panitumumab is administered to subjects by intravenous injection of <10 mL.

The radiosynthesis of ^{89}Zr-panitumumab is shown schematically in Figure 7-2. The synthesis procedure follows that reported by Vosjan, et al. (2010)[1], which is a two-step process starting from clinical grade protein and *p*-isothiocyanatobenzyl-desferrioxamine (SCN-Bz-DFO). The original method for radiosynthesis of ^{89}Zr-antibody which used the succinimidyl derivative of desferrioxamine was reported by Verel, et al. (2003)[2]. We have chosen isothiocyanato derivative of desferrioxamine because it forms a very stable thiourea linkage with lysine residue of the protein and the bioconjugation procedure is very straight forward to obtain moderate to high yield conjugate. Moreover, this chelate is commercially available with a Certificate of Analysis (COA). Clinical drug product panitumumab, manufactured by Amgen, is purchased as a drug product. Gentistic acid (2,5-dihydroxybenzoic acid), an active metabolite of salicylic acid degradation, is used during radiolabeling and in the final product purification mobile phase to protect the protein from radiolysis (Liu, et al., 2001)[3]. It is a component in the approved kit for TechneScan HDP. This preparation was recently presented at a scientific meeting[4].

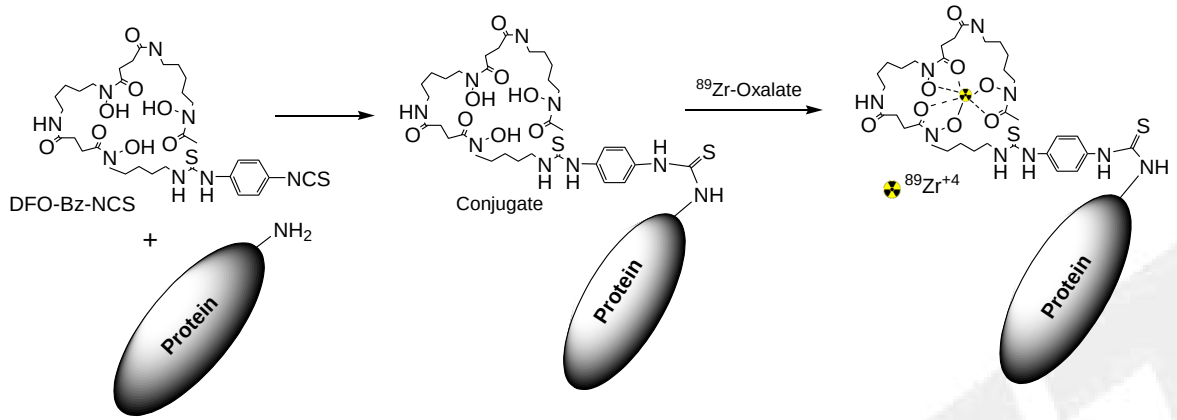


Figure 7-2. Schematic of the Radiosynthesis of ^{89}Zr-panitumumab

^{89}Zr-Panitumumab will be prepared manually in the [insert your instituation/radiopharmacy name and location here] , in accordance with USP<823> compliance standards. An ISO Class 5 lead-shielded biosafety cabinet will be used. Bioconjugation and radiolabeling will be performed in one day in a single process.

7.2 Controls for Components and Raw Materials

A complete list of forms and standard operating procedures (SOPs) are in place in the [insert your institution/radiopharmacy name here] to control the manufacturing, materials, and process from ordering to final approval for injection. A list of the documents used in the manufacture and control of ^{89}Zrpanitumumab isprovided in Section 7.15. Each reagent or supply has a unique internal specification code of three characters and a unique sequential number, for each lot for each specification code. When an item is ordered it is given a unique internal tracking number for the reagent or supply. The specification sheets list all of the reagent and supply manufacturers' addresses and contact information, including any pertinent certificate of analysis or certificate of quality criteria that these reagents/supplies must meet prior to their release for further use. Any additional testing beyond what may be listed on the certificate of analysis (C of A) or certificate of quality (C of Q) is described in this IND submission.

7.2.1 Organic Substrate Used for Bioconjugation and Radiosynthesis

The precursor panitumumab manufactured by Amgen, for sale, is the drug product approved for human administration. No further documentation is required. The purity and identity of each lot of panitumumab precursor will be confirmed by HPLC analysis. The analysis is conducted by gel-filtration HPLC using a Superdex 200 10/300 GL column with a PBS-based mobile phase (PBS-HPLC-L-) flowing at 0.80 mL/min and a column temperature of 25 to 30°C. UV absorbance and or MS detection (Figure 7-3, below) are used. This analytical separation is similar to that used for ^{89}Zr-panitumumab quality control (QC) analysis and uses the same

mobile phase to elute the precursor. The lipophilicity of the protein doesn't change upon radiolabeling. Radiosynthesis of a batch of [89]Zr-panitumumab will validate the lot of precursor because the product will have to meet all of the quality assurance criteria including HPLC radiochemical purity with the peak retention the same as the standard (protein or DFO-protein). Table 7-3, below, provides a summary of reagents, suppliers, and additional testing involved.

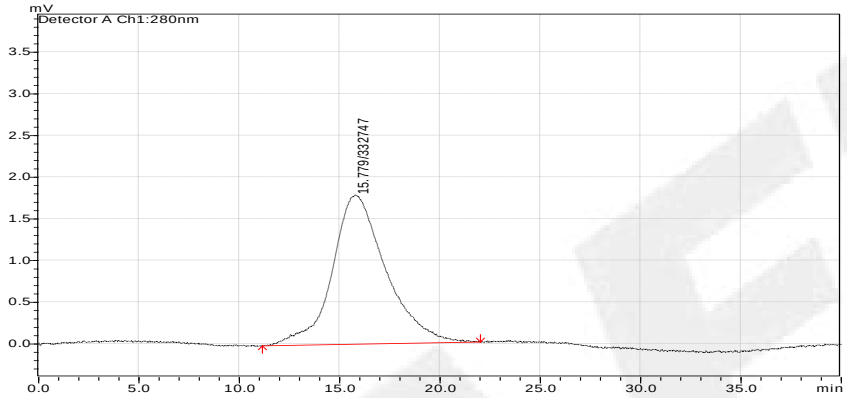


Figure 7-3. Panitumumab SS-Pani (10 µL injected of a 500 µg/mL solution), on Superdex 200 10/300 GL with PBS-NaN3-NaCl (PBS-HPLC-L-) Mobile Phase at a Flow Rate of 0.8 mL/minute

Table 7-3. Reagents, Suppliers, and Additional Testing

Reagent	Current Supplier	Additional Testing Required
Panitumumab (Vectibix®) (clinical grade)	Amgen	1. HPLC-UV or MS. UV absorbance post void volume is >90% of the protein. 2. Quality control testing on first batch required to show the correct product is made.
p-isothiocyanato-benzyl desferrioxamine	Macrocyclics, Inc.	1. MS or HPLC-UV. UV absorbance post void volume is >90%. 2. Quality control testing on first batch is required to show the correct product is made.

The purity of the chelating bifunctional ligand SCN-Bz-DFO purchased from Macrocyclics, Inc. is checked by HPLC or MS. A typical trace is shown in Figure 7-4.

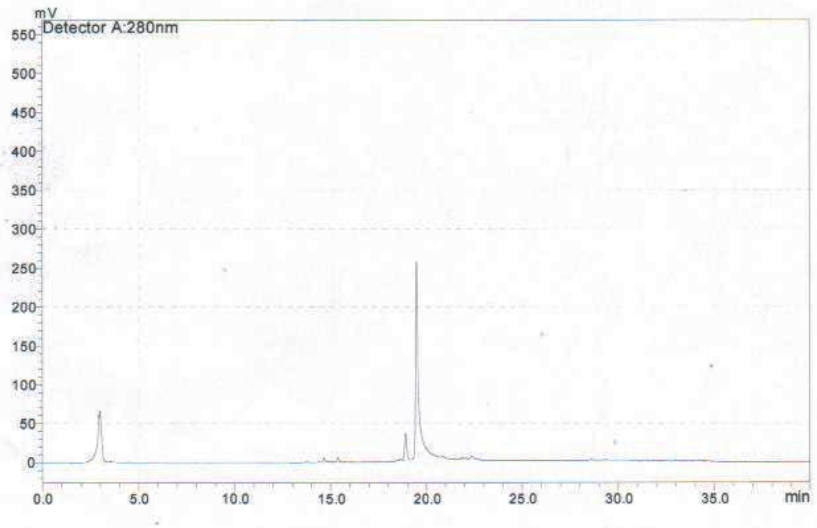


Figure 7-4. SCN-Bz-DFO (SS-DFO) on a C18 Column (Gemini, Phenomenex) with a Gradient Elution Using 90% AcCN in Water Starting 0.1 % in 0 min to 100% in 30 Minutes

7.2.2 [^{89}Zr]Zr-Oxalate Reagent

The radioactive zirconium is produced by [insert your cyclotron or vendor name here] and is delivered to the [insert your institution/radiopharmacy name here] on the same day of preparation. Production and purification procedures are described in Section 7.5.2 of this IND.

7.2.3 Other Ingredients

The reagent, 0.9% Sodium Chloride Solution, for injection, USP, used in the formulation of [^{89}Zr]-protein injection, is summarized in Table 7-4.

Table 7-4. Sodium Chloride Solution, for Injection

Reagent*	Purpose	Current Supplier
0.9% Sodium Chloride Solution, for Injection, USP (preservative free)	To make the formulation isotonic prior to injection.	Any USP supplier

* This is USP and no verification is required beyond the certificate of analysis.

7.2.4 Reagents, Solvents, Gases, Purification Columns, Solutions, and Other Auxiliary Materials

The reagents used in the bioconjugation and radiosynthesis of ^{89}Zr-panitumumab are provided in Table 7-5, below. The solutions and disposable supplies used in the radiosynthesis are provided in Tables 7-6 and 7-7, respectively.

Table 7-5. Reagents Used in Bioconjugation and Radiosynthesis of ^{89}Zr-panitumumab

Reagent	Grade	Current Supplier*
Chemicals used in the synthesis:		
Na_2CO_3	99.9% + ACS grade	Sigma-Aldrich or Mallinckrodt Baker
DMSO (Bioconjugation Step)	99.9%, anhydrous	Sigma-Aldrich or Mallinckrodt Baker
Gentistic acid	98%	Sigma-Aldrich
HEPES buffer	1.0 M Sterile pyrogen free	Sigma-Aldrich, EMD (VWR International)
Oxalic acid	99.9%	Sigma-Aldrich
0.9% Saline	Sterile and pyrogen free, USP	Any USP supplier
Sterile, Water for Irrigation	Sterile and pyrogen free, USP	Any USP supplier
Purification column and filter used in the synthesis:		
PD10	Not applicable	GE Healthcare
Sterile filter	Not applicable	Pall Corp.

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

Table 7-6. Solutions Used in the Radiosynthesis of ^{89}Zr-panitumumab

Solutions	Specification	Quantity
0.1 M Na_2CO_3	Compounded from sterile chelexed water for injection and Na2CO3 as specified in Table 7-5.	<1.0 mL
2.0 M Na_2CO_3	Compounded from sterile chelexed water for injection and Na2CO3 as specified in Table 7-5.	<1.0 mL
0.5 M HEPES buffer	Compounded from sterile chelexed Water for Injection and 1.0M HEPES buffer as specified in Table 7-5.	<1.0 mL
Gentistic acid solution (0.25 g in 100 mL saline)	Compounded from gentistic acid and 0.9% saline as specified in Table 7.5.	<50 mL
1.0 M Oxalic acid	Compounded from sterile chelexed water for injection and oxalic acid as specified in Table 7-5.	<0.2 mL
0.9% Saline	Used as purchased as specified in Table 7-5.	As needed

Table 7-7. Disposable Supplies Used in the Radiosynthesis of ^{89}Zr-panitumumab

Supply	Description	Specification	Supplier*
Sterile Vented Filter Needle	Vent for final product vial	Sterile, nonpyrogenic	International Medical Industries or equivalent
Sterilizing Product Filter 0.2 µm	Final sterilization of product before product vial	Sterile, nonpyrogenic	Pall Corp
Sterile Vent Filter 0.2 µm Millex SLGV V25 5F	Used to for venting of product vial during sterile filtration	Sterile, nonpyrogenic	Millipore Corporation
Empty Sterile Vials 10 mL borosilicate glass with gray butyl septa, sterile, nonpyrogenic	Final product vial	Sterile, nonpyrogenic	Hospira (Lake Forest, IL), SLK Abello, or Miller Analytical
Sterile Disposable metal-free pipette tips (200 µL, 1000 µL)	Used to transfer reagents during and before radiolabeling	Plastic, no silicone or latex sterile and nonpyrogenic	Bio-Rad
Sterile Disposable Syringe, 3 mL	Used to transfer reagents or solutions after radiolabeling	Plastic, no silicone or latex sterile and nonpyrogenic	Sigma Aldrich or GE Medical Systems
Sterile Disposable Syringe, 10 mL	Used to transfer product	Plastic, no silicone or latex sterile and nonpyrogenic	Sigma Aldrich or GE Medical Systems
Disposable Needles 25G x 3½"	Used to transfer reagents	Sterile, nonpyrogenic	Becton, Dickinson & Co. (Franklin Lakes, NJ)
Syringes, various sizes, 0.5 to 60 cc disposable plastic	Used for compounding and to transfer reagents	Sterile, nonpyrogenic	Becton, Dickinson & Co. (Franklin Lakes, NJ)
Disposable Needles 16G, 22G or smaller	Used for compounding and to transfer reagents product	Sterile, nonpyrogenic	Becton, Dickinson & Co. (Franklin Lakes, NJ)
Alcohol Swabs	Used to wipe septa on vials	Sterile Individually Wrapped	Various, including Cardinal Health

* These are current suppliers. If the equivalent grade of supply can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

7.3 Reference Standards

The reference standards provided in Table 7-8, below, are used in the quality control methods of ^{89}Zr-panitumumab injection.

Table 7-8. Reference Standard Compounds

Reagent/Supply	Chemical Specification	Current Supplier*
Panitumumab	As supplied for clinical use, checked in-house for purity by product analytical HPLC UV to be >95% pure	Amgen
DFO-Panitumumab conjugate	95% purity or greater, checked by analytical HPLC (Figure 7-5)	Prepared in [insert your institution name here]

*These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

DFO-panitumumab conjugate is prepared in the [insert your institution/radiopharmacy name here] following standard SOP (**MPR-^{89}Zr-PAN**-). The conjugate concentration is determined by Lowry Assay (**your institution/radiopharmacy name-Q116),** and DFO to Mab molar ratio is determined by ^{89}Zr binding assay (**your institution/radiopharmacy name-Q120**). This freshly prepared conjugate is then used to develop standard calibration curve for the determination of DFO-protein concentration in every batch. The chromatogram (Figure 7-5, below) shows that, although small, the minimum standard concentration needed <26 µg/mL can be detected with sufficient signal to noise for quantification of the UV absorbance. Figure 7-6 illustrates the standard calibration curve obtained in the different concentrations (26 µg/mL – 210 µg/mL) of DFO-panitumumab.

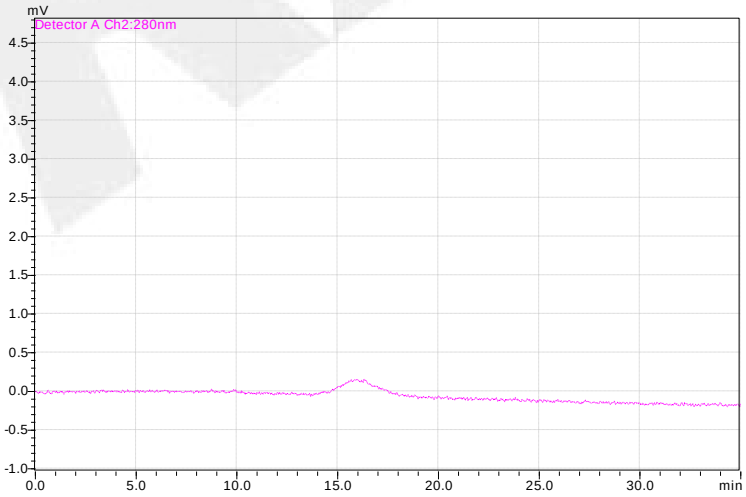


Figure 7-5. Elution/Purity of DFO-panitumumab (10 µL injected of a 26 µg/mL solution), Using PBS-NaN3-NaCl (PBS-HPLC-L-) Mobile

Phase and Superdex 200 10/300 GL Column with Flow Rate of 0.8 mL /minute

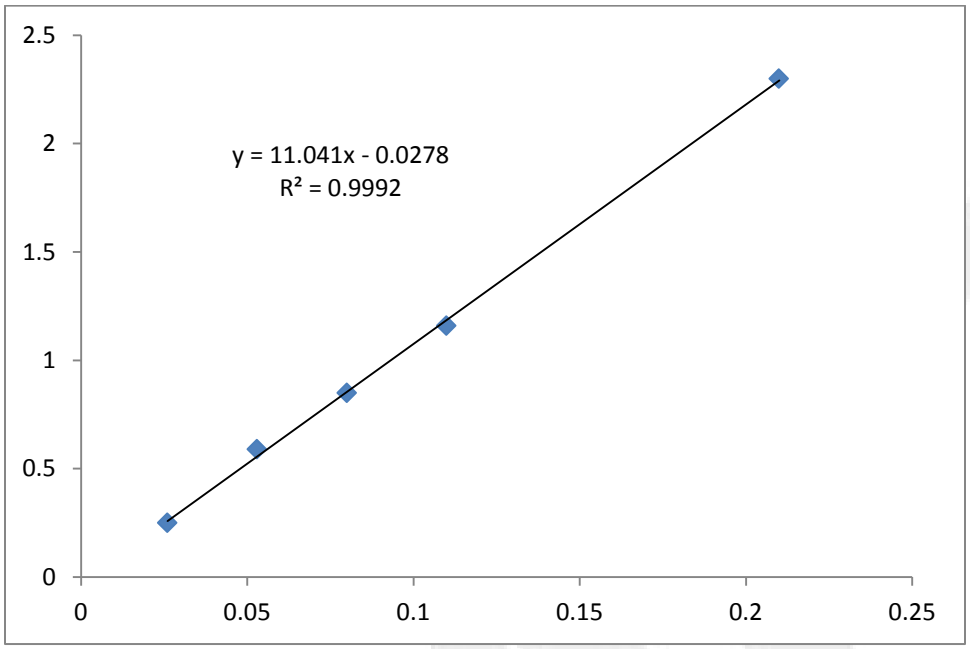


Figure 7-6. Standard Calibration Curve Obtained in the Different Concentrations (26 µg/mL – 210 µg/mL) of DFO-panitumumab (X-axis is for concentration (mg/mL) and Y-axis is for normalized UV peak area (Y X 10^5) of HPLC chromatograms)

7.4 Manufacturing and Testing Facilities

The IND Sponsor will maintain responsibility for oversight of quality assurance and will work closely with the principal chemistry investigator to ensure correct implementation of the written SOPs, Master Production Record, Master Batch Record, Master Compounding Records, Specification Sheets, Forms, and all other written documentation that is not specifically referenced here.

The ^{89}Zr-panitumumab will be manufactured and tested at the following site under the direction of the [Insert your institution information here].

Name: **Insert name of facility with responsible person**
Principal Chemistry Investigator:
Registered Nuclear Pharmacist:

Address: **Insert name and address of facility**

Email: **Phone:** **Fax:**

7.5 Manufacture of the Drug Substance

7.5.1 General Description of the Method for Preparation of the ^{89}Zr-panitumumab

The general chemical schematic of the radiosynthesis preparation of ^{89}Zr-panitumumab is illustrated in Figure 7-2, above. The general process for radiosynthesis of ^{89}Zr-panitumumab is also shown as a flow chart in Figure 7-7, below. In brief, 8-10 mg of protein (**SS-Pani**) is incubated with 3 equivalents of SCN-Bz-DFO (**SS-DFO**) for 1 hour at 37°C. The conjugate is then purified on a PD10 size exclusion chromatography column (**SS-PD10**) using 0.9% saline (USP) as mobile phase. Concentration (mg/mL) of the conjugate is determined by HPLC (**your institution/radiopharmacy name-Q119**). In another reaction vial, ~4.0 mCi of ^{89}Zr-oxalate (**SS-^{89}Zr-oxa**) solution in 1.0 M oxalic acid (~200 µL) is neutralized by 2 M Na_2CO_3 solution (**NaCO-L-2-**). To this reaction vial is added 1-1.2 mg of conjugated protein, 0.2 mL of gentistic acid solution (**GenA-L-**), and 0.5 mL of 0.5 M HEPES buffer (**HEPES-L-**). The reaction mixture is incubated for 1 hour at room temperature. The crude product is purified on a PD10 column using gentistic acid solution (**GenA-L-**) as mobile phase. Purified product (~2 mL) is diluted to 5 mL with 0.9% saline and transferred to a 10-mL vented sterile vial through a sterile filter (**SS-SPF-protein**).
The preparation (bioconjugation, radiolabeling, and purification) takes 5 hours. The decay corrected radiochemical yield for the qualification runs was 75.0 ± 5.0% (n=3). Purified drug substance is collected through a 0.22 µm sterilizing filter into a vented sterile vial. Samples are removed for analysis of product quality (see IND Section 7.6).

The radiosynthesis of ^{89}Zr-panitumumab is described in greater detail in the ^{89}Zr-panitumumab Master Batch Record MPR-^{89}Zr-PAN provided at Section 7.15. The control information for each batch is captured in a copy of the Master Batch Record (MBR) that is assigned a unique batch number for every production run. The entire preparation is performed inside an ISO Class 5 lead shielded biosafety cabinet as illustrated in Figure 7-8.

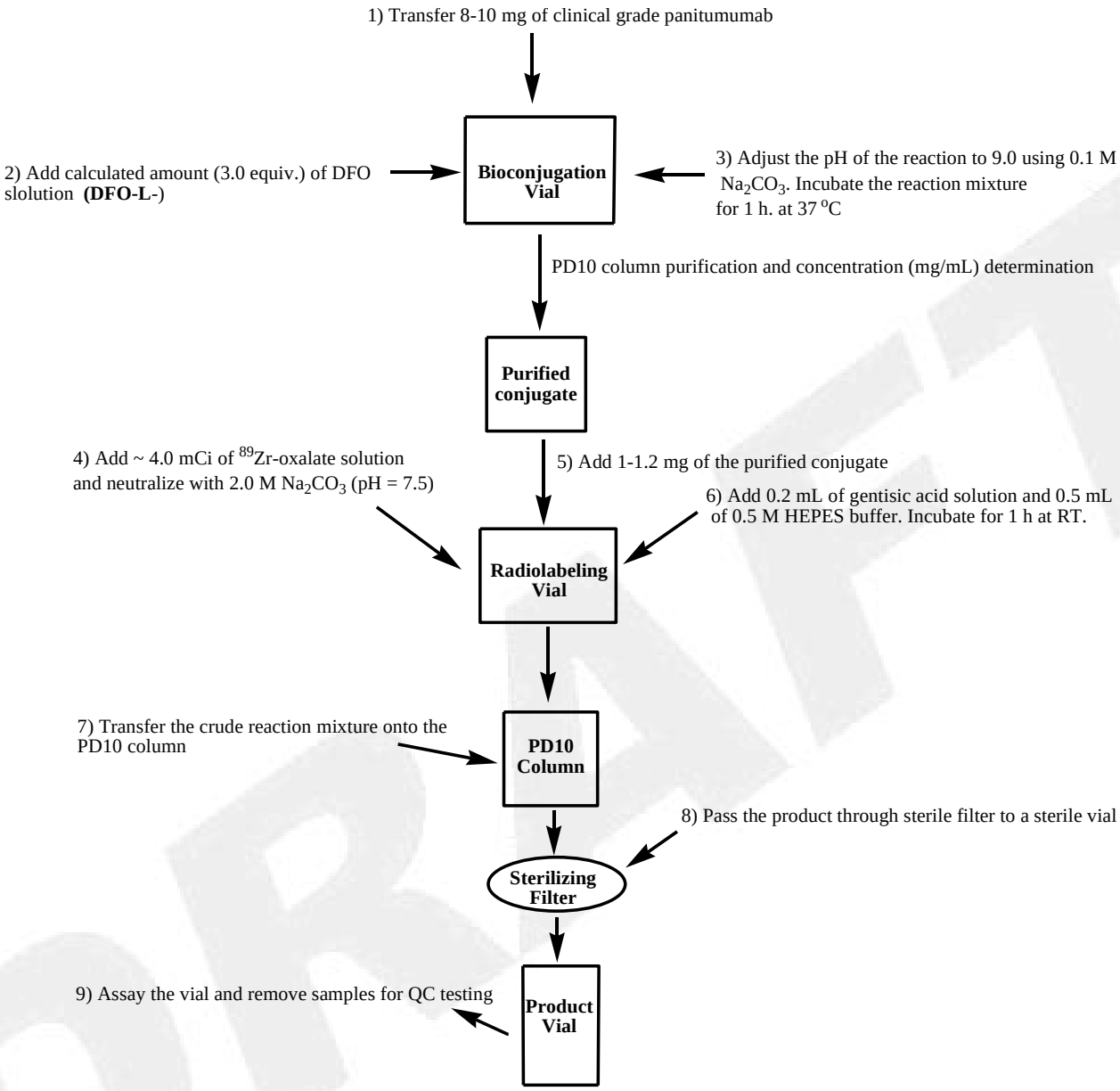


Figure 7-7. Schematic Flow Chart of the Process for Radiosynthesis of ^{89}Zr-panitumumab

Figure 7-8. ISO Class 5 Lead Shielded Cabinet Where ^{89}Zr-panitumumab Will Be Synthesized

7.5.2 Cyclotron Production of [^{89}Zr]Zr-oxalate

[^{89}Zr]Zr-oxalate is produced by the cyclotron facility at [insert your cyclotron or vendor name here] . Pressed pellets of yttrium metal mesh (200 mg, 4N purity, American Elements) are irradiated with a proton beam of 14 MeV and a current of 20 μA for 2-5 hours on an in-house beam-line on a GE PETtrace cyclotron. The beam-line and target setup consist of an aluminum holder for a target cup and degrader foil at the end of a 16-inch long aluminum tube mounted to a vacuum exit port of the GE PETtrace (Szajek et al., 2007)[5]. Figure 7-9 shows a close-up of the pressed pellet under an aluminum cover foil as well as the water cooling supplied to the back of the target cup. Helium-cooling flows over the face of the degrader foil and exit foil through the aluminum holder. A collimator is employed at the end of the evacuated aluminum tube to aid in centering the beam. Total beam current is measured at the back of the target cup holder. A 0.01-inch aluminum foil is used to degrade the incident beam energy to less than 14 Me protons. The production rate is 1.11 ± 0.07 mCi/μA-h (n=10). Purified ^{89}Zr is obtained in 92% radiochemical yield with less than 0.40% ^{88}Zr at EOB. Typically, a 3-hour run at 20 μAmps yields 30 mCi of ^{89}Zr in 1.0 mL of 1.0 M Oxalic acid after processing the following day.

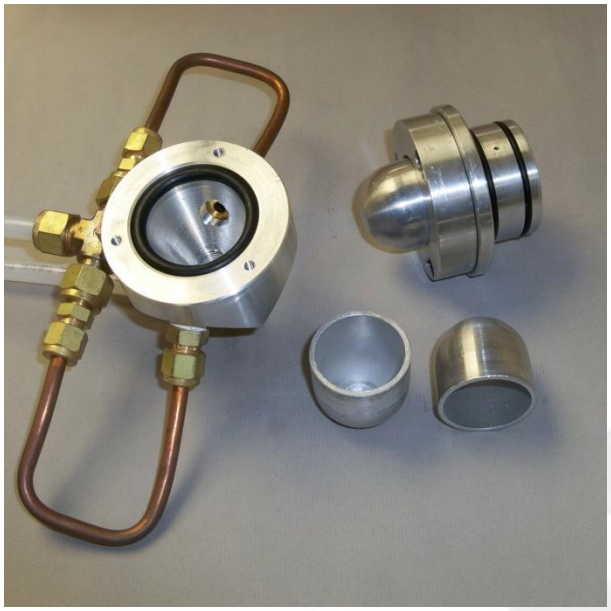


Figure 7-9. GE PETtrace Solid Target Cup Components Used in ^{89}Zr production

Zirconium-89 is separated as [^{89}Zr]- Zirconium-oxalate from the irradiate yttrium mesh using the method of Lewis and co-workers (2009)[6]. A modular panel used for the processing of the zirconium-89 radioisotope is shown in Figure 7-10. Briefly, to a mini-vial charged with the irradiated metal is carefully added 6N HCl (4 x 0.5 mL) and ultra-pure H_2O_2 (100 μL, Fluka). The resulting solution is warmed to 90°C before dilution with 18 mΩ H_2O (5 mL) and loading onto a pre-washed column of hydroxamate resin (200 mg). The resin is washed with 2N HCl (4 x 2.5mL) followed by 18 mΩ H_2O (4 x 2.5 mL). Zirconium-89 is eluted with 1.0 M Oxalic acid (4 x 0.5 mL and 2 x 1.0 mL) in greater than 96% radiochemical yield. The [^{89}Zr]Zr-oxalate yield for a 3-hour irradiation was 66.3 ± 4.1 mCi (n=8) at EOB. Specific activity of the isolated Zr-89 in 1M oxalic acid is greater than 456 ± 94 Ci/mmol (n=8), suitable for radiolabeling of peptides and antibodies. The radioisotope is analyzed by GeLi gamma spectroscopy prior to shipment. Less than 0.2 % ^{88}Zr is present at the EOS.

Figure 7-10. ^{89}Zr Purification Set-Up to Produce Pure ^{89}Zr-oxalate

7.5.3 [^{89}Zr]Zr-oxalate

The [^{89}Zr]Zr-oxalate obtained from [Insert your cyclotron or vendor name here] is diluted to the specific concentration ~20 mCi/mL with 1.0 M oxalic acid (**Oxa-L-**).

7.5.4 Synthesis of ^{89}Zr-panitumumab

A total of 8-10 mg of panitumumab is incubated with 3 equivalents of bifunctional chelate SCN-Bz-DFO at 37°C for 1 hour. The pH of the reaction is adjusted to ≈9 using 0.1 M Na_2CO_3. The conjugate is separated from free chelate by size exclusion chromatography on a PD10 column with 0.9% saline elution. Both literature reports (Vosjan et al. 2010)[1] and our radiometal binding assay (**your institution/radiopharmacy name-Q120**) of the purified conjugate have demonstrated that 3 equivalents of chelate is sufficient to keep the chelate to protein labeling ratio (Figure 7-11) within the desirable range (1.4 ± 0.2, n=6).

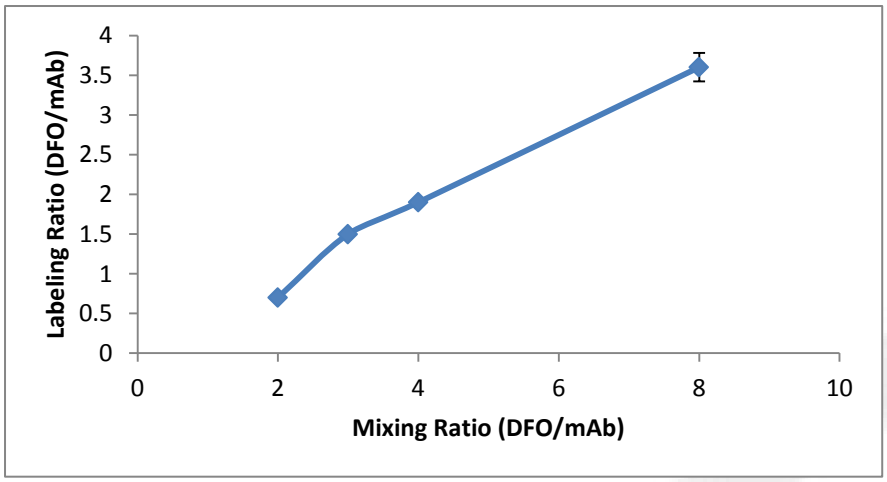


Figure 7-11. Chelate (DFO) to Protein (mAb, panitumumab) Molar Labeling Ratio Measured at Various Chelates to Protein Mixing Ratios

A total of 1-1.2 mg of the conjugate is added to a reaction vial with ≈ 4 mCi of ^{89}Zr-oxalate in presence of gentistic acid and HEPES buffer. The reaction mixture is incubated for 1 hour at room temperature. Radiochemical purity of the product is checked by radio-ITLC (**your institution/radiopharmacy name-Q322**). The schematic of the radiosynthetic method for ^{89}Zr-panitumumab is repeated below.

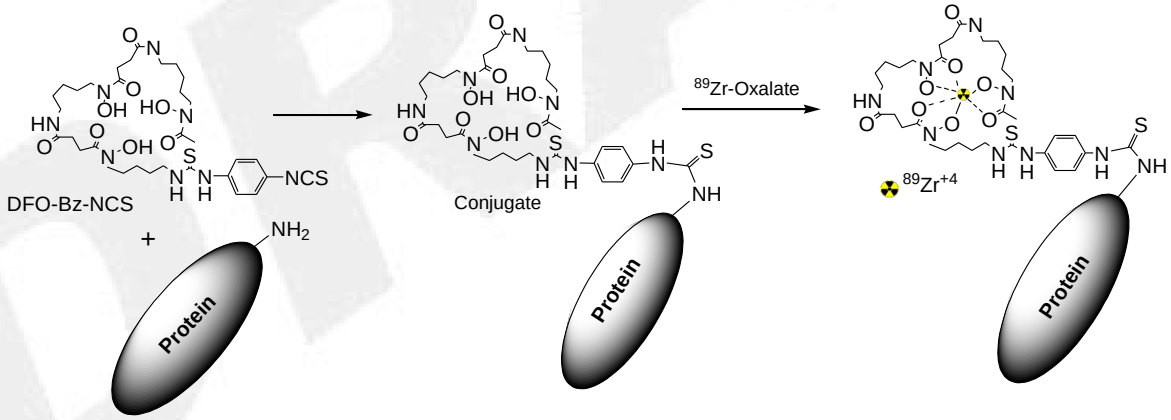


Figure 7-12. Schematic of Radiosynthetic Method for ^{89}Zr-panitumumab

7.5.5 Purification of ^{89}Zr-panitumumab

A PD10 column is equilibrated with 20 mL of mobile phase (**GentA-L-,** 2.5 mg gentistic acid/ml in 0.9% saline) and the product is purified on this PD10 column and fractions of 0.5 mL, 1.5 mL, and 0.5 mL are collected. Radioactivity of each fraction is measured in a dose calibrator. On the fraction collection, the early fraction with very low amount of radioactivity is discarded. The

middle fraction (1.5 mL) has the pure product, and the last fraction can have pure product. Fractions 2 and 3 with high activities are combined together after checking radiochemical purity of each by ITLC. The total volume of combined fractions is usually 1-2 mL. Product is diluted to 5 mL with 0.9% saline (USP). The chemical purity of the product is checked by ITLC and HPLC. The decay-corrected radiochemical yield for the qualification runs was 75.0 ± 5 % (n=3). The ^{89}Zr-panitumumab is sterilized by passing through a 0.22 µm sterilizing filter into a vented sterile vial and the filter is bubble tested after use. Samples are removed for analysis of product.

This procedure is detailed in the ^{89}Zr-panitumumab: Master Batch Record **MPR-^{89}Zr-PAN** included in Section 7.15.

7.6 In Process Testing Quality Assurance

Rapid in process testing is performed to check the purity and concentration of the conjugate before radiolabeling. Analytical HPLC (**your institution/radiopharmacy name**-Q320) of the purified conjugate demonstrates the chemical purity and concentration by using standard calibration curve (**your institution/radiopharmacy name**-Q119). Chemical purity determined by HPLC is usually >99% and concentration is around 4-5 mg /mL. In HPLC chromatogram no high or low molecular weight UV peak should be observed, which indicates that there is no fragmented or aggregated byproduct of protein generated during the process.

7.7 Post Synthesis Processing and Quality Assurance of the ^{89}Zr-panitumumab

7.7.1 Sampling for Quality Assurance and Storage

The drug product is assayed for total radioactivity and is examined for particulates. The integrity of the sterilizing filter is tested. Two samples totaling at least 300 µL are removed for measurement of pH, analytical HPLC measurements of specific activity, radiochemical and chemical purity, radionuclidic purity by half-life determination, apyrogenicity, and sterility of the product. At least 100 µL of the sample is retained for further testing, if necessary. The product dose is drawn, labeled, and after all tests except sterility have been passed, the product dose is released for injection. If the PTS chromogenic endotoxin test is used, then the endotoxin test must be completed before release of the product. If the Limulus amebocyte lysate gel clotting method is used to determine endotoxin level, then a 20-minute endotoxin test may be used to release the dose but a 60-minute endotoxin test must be completed. The sample for sterility testing is inoculated within 48 hours.

7.7.2 Master Production and Master Batch Records

[Insert your institution/radiopharmacy name here] conducted 3 qualification runs. The information collected from these qualification runs was used to optimize the language of the Master Production and Master Batch Records but no substantial changes were required. See IND Section 7.10 for additional information on the qualification runs. These optimized procedures will be used at the [Insert your institution/radiopharmacy name here] for production of ^{89}Zr-panitumumab for human use.

7.7.3 Reprocessing of PET Drug Product

The final ^{89}Zr-panitumumab drug product will be reprocessed for only one reason. The final drug product may be re-sterilized once in the event of a sterilizing filter integrity test failure.

7.8 Container/Closure

The drug substance is manufactured inside the ISO Class 5 lead-shielded cabinet using sterile vials and pipette tips. USP grade saline is used for dilution. The drug substance is then sterile filtered into the final drug product vial, the sterile USP Type I, Glass, Gray Butyl Rubber Stopper, Vial. An integrity bubble point test is performed on the sterilizing filter, in addition to performing an endotoxin and a sterility test on the final drug product.

7.9 Controls for Finished Drug Product

The quality control oversight will remain at [Insert your institution/radiopharmacy name here]. Several quality control tests are run on the ^{89}Zr-panitumumab product prior to release for human administration, to assure the quality of the final product. The quality control tests that are run are summarized in Table 7-9. They are consistent with the guidelines for USP<823> Radiopharmaceuticals for Positron Emission Tomography-Compounding.

Table 7-9. Quality Control Tests for the ^{89}Zr-panitumumab Product

Quality Control Test	Description	Requirements For Pass	Test Must Pass Prior To Product Release
Chemical Purity	Visual inspection for color and particulates	Clear and Colorless	yes
Filter Integrity	Test bubble point	Meet pressure specified by manufacturer	yes
pH	pH per USP<791>	pH must be between 6 and 8	yes
Chemical and Radiochemical Purity	HPLC consistent with guidelines of USP<621>	Radiochemical Purity: >95% Chemical Purity: DFO-PAN <1 mg/dose	yes
Radiochemical Purity	TLC	Rf <0.5 and Purity >90%	yes
Radionuclidic Purity	Half-life Determination	74-82 hours	yes
Specific Activity	Test strength	≥1.5 mCi/mg of mAb	yes

Quality Control Test	Description	Requirements For Pass	Test Must Pass Prior To Product Release
Bacterial Endotoxin Levels	Limulus Amoebocyte Lysate (LAL) by gel clot or PTS	<175 EU per dose	yes
Sterility	USP sterility test (USP <71>)	No growth observed in 14 days	No

All of the quality control tests will be completed prior to product release except for the USP sterility test. In the event of a positive sterility test result, action will be taken within 24 hours. The treating physician will be notified with a summary from the principal physician investigator at the investigational site and a report will be sent to the IND sponsor.

If all tests, with the exception of the pending sterility test, are within acceptable limits, the product will be released for administration to the subject. If one or more of the quality assurance tests do not meet the required specifications listed above, then one of two actions will be taken. For all tests with the exception of the filter integrity and half-life tests, if the test does not meet the specifications listed above, the product will be failed and will not be released. As a result of this failure an Out-of-Specification Investigation will be conducted to determine the cause of the aberrant test result. If the 0.22 µm filter used to filter the final product fails the integrity test both initially and upon re-wetting of the filter then the final product will be re-sterilized using a new sterile filter. If the half-life determination does not meet specifications, it may be due to insufficient time points. In this case, the dose is not to be released and the half-life determination may be repeated or more time points may be collected for the determination to improve the counting statistics. If the half-life determination passes after the second set of counts, then the dose may be released and an Out-of-Specification Investigation will be conducted as well. If the half-life determination does not pass after the second set of counts, the product will be failed and will not be released.

7.10 Analytical Test Procedures

7.10.1 Chemicals for Quality Control Analyses (QC)

The reagents and solutions used for quality control analyses are provided, below, in Tables 7-10 and 7-11, respectively.

Table 7-10. Reagents Used for Quality Control Analyses (QC)

Reagent	Chemical Grade	Current Supplier*
Reagents used for more than 1 test:		
Water for injection (WFI)	Sterile, pyrogen free, USP	Any USP supplier
Water for HPLC	HPLC Grade	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
pH:		
pH buffers	any NIST traceable buffer	VWR
ITLC:		
Citric acid	>99.5% ACS reagent	Sigma-Aldrich and Mallinckrodt Baker
Na2CO3	>99.5% ACS reagent	Sigma-Aldrich and Mallinckrodt Baker
Chelex resin	200-400 mesh, bio-reagent grade	Bio-rad
***HPLC mobile phase in water*:**		
NaN3	>99.5%, ACS reagent	Sigma-Aldrich or Mallinckrodt Baker, Inc.
PBS 10 M	ACS bio-reagent	Sigma-Aldrich or Mallinckrodt Baker, Inc.
NaCl	>99.8% ACS reagent grade	Sigma-Aldrich, Mallinckrodt Baker, Inc., or VWR Scientific
Bacterial Endotoxin Test:		
Control Standard Endotoxin (CSE)	as supplied	Associates of Cape Cod, Charles River or Cambrex
Limulus Amoebocyte Lysate (LAL) single test vials	as supplied	Associates of Cape Cod, Charles River or Cambrex
Limulus Amoebocyte Lysate (LAL) portable test system cartridges (PTS)	as supplied	Charles River
LAL Reagent Water	as supplied according to USP/NF	Associates of Cape Cod, Charles River or Cambrex

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

Table 7-11. Solutions Used for Quality Control Analyses (QC)

Solutions	Grade of Chemicals
HPLC Analytical Mobile Phase, 5 mL PBS 0.05M, 30 mL NaCl 0.15M, and 0.65g of NaN3 in 1L of HPLC Grade Water (v:v)	HPLC grade water as listed above
The preparation of ITLC mobile phase, 0.42 g citric acid and 1 mL of 2 M Na2CO3 dissolve in 100 mL of chelex water	All reagents used as listed above
Chelex water, HPLC grade water passed though the chelex resin	All reagents used as listed above

7.10.2 Materials for Quality Control Analyses (QC)

The supplies used for quality control analyses are listed in Table 7-12, below.

Table 7-12. Supplies Used for Quality Control Analyses (QC)

Supply	Purpose	Current Supplier*
pH strips, BDH pH test Strips (4.5-10)	Assay of pH	VWR
TLC plates, Backed	ITLC analysis	Auburn Biostrips, CA
Analytical Column: Superdex200 10/300 GL	HPLC analysis for chemical and radiochemical purity	GE Healthcare

* These are current suppliers. If the equivalent grade of chemical can be obtained from other suppliers as suppliers change, alternate suppliers will be used.

7.10.3 Validated Standard Test Procedures

All of the analytical test procedures are performed using high quality solvents and reagents, which have been carefully logged in, controlled, and verified in the same manner as the reagents for the manufacturing process. Testing forms have been developed to ensure consistency for documentation of the collected test information.

Particulates: The [89]Zr- product solution is examined visually. The chemical purity by visual inspection is straightforward, the final drug product in the vial should be clear and colorless without any visible particulates as per USP <823> and USP <631> Color and Achromicity. The product must pass this test in order to be released.

Filter Integrity: Because the USP sterility test requires 14 days to complete, the [89]Zr-panitumumab product solution sterility cannot be assured prior to injection. In addition to working in an ISO Class 5 environment when preparing the solutions used in manufacture of the [89]Zr-panitumumab and the final product vial, the [89]Zr-panitumumab is passed through a 0.2 µm

sterilizing filter into the final product vial. After the ^{89}Zr-panitumumab is collected, the sterilizing filter is tested for filter integrity to give an indication of likelihood of product sterility. Filter integrity is tested in a bubble point procedure, whereby the sterilizing filter is placed on a gas line with a pressure gauge and the outlet of the filter is placed under water. The gas pressure on the inlet to the filter is increased slowly until a steady stream of bubbles is observed at the filter outlet. The pressure when the bubble stream begins is recorded and compared with the manufacturer's pressure rating for the filter, from the certificate of quality. If the observed bubble point pressure exceeds the manufacturer's specifications, the filter integrity test is passed. If the bubble point pressure is too low, the filter may be rewetted and tested again. If the bubble point pressure is greater than the manufacturer's specifications, the test is passed; if not then the product must be re-sterilized by passing through a second sterilizing filter. That filter must then pass the bubble point test. The product ^{89}Zr-panitumumab may only be re-sterilized once. This test must be passed for an ^{89}Zr-panitumumab dose to be released.

pH: Because the product volume is small and because the product is radioactive, instead of a pH meter, pH test strips are used. The pH test strips are checked by pipeting pH 5 and pH 7 calibrated commercial pH standards onto individual strips. The color on the strips must match the pH 5 and pH 7 on the color key supplied with the test strips. Then the ^{89}Zr-panitumumab is pipetted onto another test strip and the color checked against the color key. The result is written down and the measured pH must be between pH 6 and pH 8 for the product ^{89}Zr-panitumumab to be released.

Chemical and Radiochemical Purity: HPLC chromatography analysis will be used to determine purity for this drug product. The final radiochemical purity is proposed to be greater than or equal to 95%. As the preparation process can be completed under very mild conditions nonradioactive UV impurities are not generated. In HPLC analyses, no UV peaks are observed before and after the ^{89}Zr-panitumumab peak. Both chemical and radiochemical purity are measured by UV absorbance at 280 nm and radiation peak of the ^{89}Zr-panitumumab eluted from a Superdex 200 gel filtration column (GE Healthcare). The column flow rate is 0.8 mL/minute and is kept at room temperature, 25 to 30°C. The typical retention of ^{89}Zr-panitumumab is between 16 to 18 minutes for the UV absorbance and the radioactivity ~0.8 minutes further downstream from the UV detector. The standard concentrations must bracket the sample or bracket the minimum acceptable mass limit. All standards must be baseline resolved (resolution >1.5) for a valid analysis. A linear regression is determined for UV absorbance peak areas of the standards (DFO-protein). This constitutes the calibration curve. Then the peak area of the ^{89}Zr-panitumumab drug product is fit on the calibration curve to determine the protein concentration in the drug product sample. Typically the final concentration of the DFO-protein in the ^{89}Zr-panitumumab product to be tested will be <200 µg/mL and the protein mass will be <1 mg per dose. No low or high molecular weight UV impurities were found in the qualification runs.

Figure 7-13, below, shows a typical HPLC chromatogram of ^{89}Zr-panitumumab. Figure 7-14 depicts a typical HPLC chromatogram of ^{89}Zr-panitumumab with co-injection with standard DFO-protein.

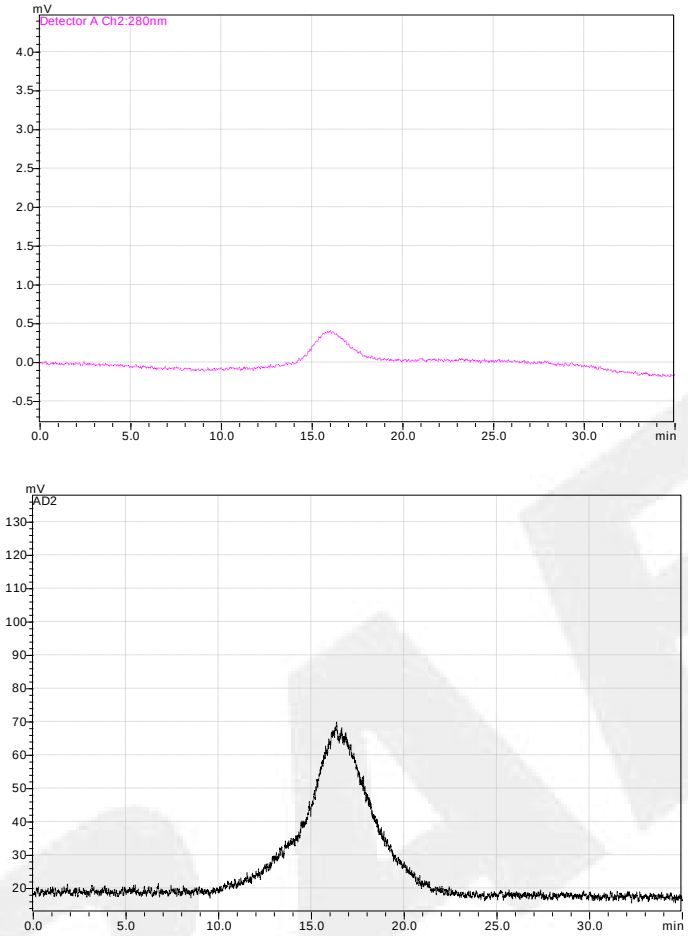


Figure 7-13. Typical HPLC Chromatogram of ^{89}Zr-panitumumab. Top trace is the UV absorbance at 280 nm. Radiation peak is shown in the bottom traces. The top trace is expanded so that UV impurities can be seen if present. HPLC is performed on a Superdex200 10/300 column using standard mobile phase (PBS-HPLC-L-) with flow rate 0.8 mL/minutes at 280 nm.

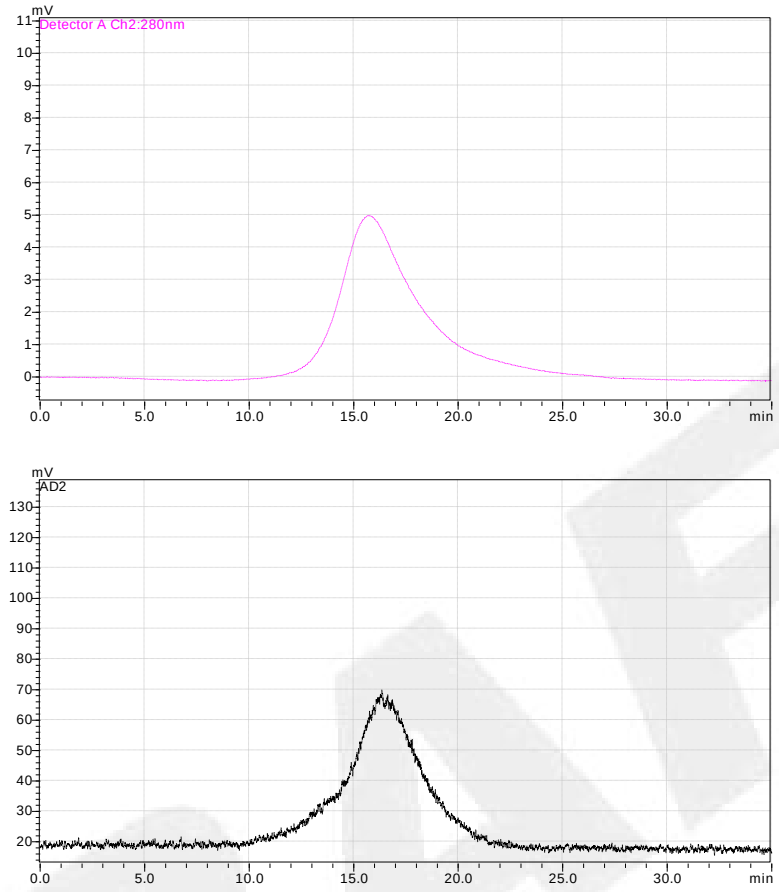


Figure 7-14. Typical HPLC Chromatogram of ^{89}Zr-panitumumab with Co-injection with Standard DFO-Protein. Top trace showed UV absorbance at 16.5 minutes at 280 nm and bottom trace showed radiation at 17.2 minutes. There is tubing between the UV and radiation detectors so that the ^{89}Zr-panitumumab is eluted at 16.5 minutes for UV and 17.2 minutes for radiation.

Two PD10 purification steps are associated with this preparation from GMP grade protein panitumumab. In the first purification step, DFO-protein conjugate is separated from excess DFO. Typical yield of the in-process intermediate conjugate is ~75%. An appropriate quantity of this purified conjugate (~1.0 mg, ~200 µL) is immediately used for ^{89}Zr labeling. The labeled DFO-protein is purified on a PD10 column (second purification step) to separate ^{89}Zr-panitumumab from excess ^{89}Zr-oxalate. An ITLC test of the purified ^{89}Zr-panitumumab shows >95% radiochemical purity (R_f <0.5). In ITLC, unbound ^{89}Zr-oxalate and free DFO bound ^{89}Zr move with the solvent front (R_f >0.5), and only ^{89}Zr bound with protein stays at the origin (R_f <0.5). The >95% radiochemical purity by ITLC means there is little or no DFO chelate impurity present in the final product. The radiochemical purity is further checked by HPLC. In HPLC chromatogram no high or low molecular weight radiation or UV peaks are observed, indicating that no fragmented or aggregated byproducts of protein are generated during the manufacturing. Between 7-10 µL of DMSO solution of DFO is used in the bioconjugation step. After passing through two purification steps, the possibility of the presence of this solvent in

the final product is very low. Moreover, as per FDA Guidance for Industry ICH Q3C,[7] DMSO is a Class 3 solvent that the FDA recommends be limited to less than 50 mg/day in pharmaceuticals. Therefore, even if all of the DMSO remained in the final product, it is a trace amount, well under the guidance.

Radiochemical Purity and Identity: The use of Radio-Thin Layer Chromatography to determine the radiochemical purity and identity were validated using HPLC citric acid solution as mobile-phase. The R_f values obtained from these studies were below 0.5 for the 3 qualification runs. The limit has been set at R_f <0.5 for the final ^{89}Zr-panitumumab because the TLC test is included only to separate unbound ^{89}Zr (solvent front) from the product (Figure 7-15). The specification for the purity is greater than or equal to 90% using this methodology. This is primarily a test for free ^{89}Zr and ^{89}Zr bound in free DFO. If present, both of these will move with the solvent front with an R_f value equal to >0.5, so this is an adjunct test to the analytical HPLC. The identity is confirmed by HPLC co-injection of the nonradioactive DFO-protein standard with the drug product to confirm the retention time values are consistent (Figure 7-14).

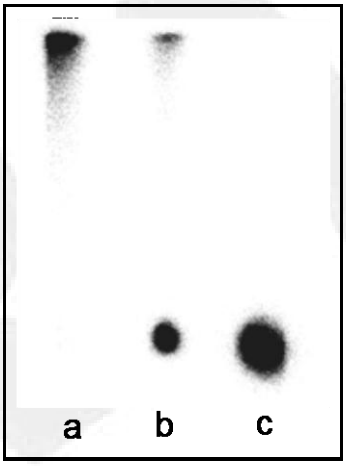


Figure 7-15. Phosphor Images of Radio-ITLC Strips
a. ^{89}Zr-oxalate (control); b. ^{89}Zr-DFO-panitumumab before purification; c. purified ^{89}Zr-DFO-panitumumab

Radionuclidic Purity: This test is used to determine the identity of the radioactive nuclide and should not vary with the compound being tested. For the test, an aliquot of the product ^{89}Zr-panitumumab is counted in an ion chamber or gamma counter at least 5 times. The half-life of the radioactivity is determined for each activity measurement using the following equation.

$$T_{1/2} = \frac{\ln(2)\ x\ (t_x - t_0)]}{(ln\ A_0 - lnAt_x)}$$

Where $\mathbf{A_t}$ is the activity (background subtracted) measured at each time-point (other than time zero).

$\mathbf{A_0}$ is the activity (background subtracted) measured at time-zero.

t is the time in units of minutes.

$\mathbf{t_{½}}$ is the calculated half-life in units of minute.

The half-life test result for ^{89}Zr must be between 74 and 82 hours for the dose to pass. The USP radioactivity general chapter <821> states that the half-life can be "readily determined by successive counting of a given source of a radionuclide over a period of time that is long compared to its half-life". The variation used here is to count for only a fraction of the half-life of 78 hours. To count a sample for over 78 hours would reduce the radiopharmaceutical dose by over half and might only provide somewhat better counting statistics.

Bacterial Endotoxin: Levels are tested and qualified using one of two procedural methods; both are based on the USP recommendations, but using control standard endotoxin referenced to USP RSE. Either a gel clot method is used with single test vials or the portable test system from Charles River Laboratories. All of the bacterial endotoxin levels were <175 EU per batch for the initial qualification syntheses.

Sterility: Sterility is tested using the direct inoculation method as is required by the USP. The test is completed after release of the product.

7.10.4 Results of ^{89}Zr-panitumumab Qualification Runs

All of the three completed qualification ^{89}Zr-panitumumab radiosyntheses passed all the acceptance criteria. The results are summarized in Tables 7-13 and 7-14, below. An example of the Final Product QC Test results is provided in Section 7.15.

Table 7-13. Summary of Measured Particulates, Filter Integrity, pH, Endotoxin and Sterility for ^{89}Zr-panitumumab from [Insert your institution/radiopharmacy name here] Qualification Runs

Batch Number MBR-^{89}Zr-PAN-	Particulate Test	Filter integrity test (PSI)*	pH	Half-life (min)	Measured Endotoxin (EU/mL)**	USP Sterility Test (14 day)
04-17-12-001	clear and colorless	56	7.0	76.3	<2	pass
04-18-12-001	clear and colorless	55	7.0	76.0	<2	pass
04-25-12-001	clear and colorless	58	7.0	76.4	<2	pass

*Manufacturer specified pressure rating was >46 psi.
**A 1/40 dilution is used to avoid interference from the salts so <2 is equal to 40 X <0.05 EU/mL, the lowest sensitivity for the PTS cartridges that was used.

Table 7-14. Summary of the Measured ^{89}Zr-panitumumab Radiochemical and Chemical Purity from [Insert your institution/radiopharmacy name here] Qualification Runs

Batch Number	TLC		HPLC		
MBR-^{89}Zr-PAN-	R_f	Radiochemical Purity (%)	Radiochemical Purity (%)	Protein (µg per 1.5 mCi dose)*	Protein (µg/mL)**
04-17-12-001	0.48	99	100	450	150
04-18-12-001	0.46	99	100	520	180
04-25-12-001	0.48	98	100	500	220

*Time of dose calculation is 1 hour post end of synthesis.
**Concentration is measured after sterile filtration.

The dependence of the volume of the product that is used upon the amount of radioactivity in the product is the reason that we have specified the total µg of materials in a dose as the criteria for release of product, rather than a µg/mL as acceptance criterion. From the qualification runs, if 1.5 mCi of product were used, the protein in the product would be ≈480 ± 30 µg (average ± standard deviation for the 3 qualification runs). All doses will be brought to a final volume in between 5-10 mL for injection using sterile saline for injection, preservative free, USP.

7.11 Immunoreactivity and Specificity of ^{89}Zr-panitumumab

The immunoreactivity of the ^{89}Zr-panitumumab was assessed in a radioimmunoassay, as detailed in the published literature (Nayak et al. 2012)[8], using methanol-fixed cells. In brief, serial dilutions of ^{89}Zr-panitumumab (≈200,000—30,000 cpm in 50 µL of BSA/PBS) were added to small test tubes containing MDA-MB-468 cells (1X10^6/50 µL of BSA/PBS) from NCI 60 cell screen. Following 2 hour incubation at 37°C, the cells were washed, pelleted, and counted in a γ-scintillation counter. The percentage of binding was calculated for each dilution and averaged. It was observed that 65% ± 5% of the radioactivity was bound.

The specificity of the radiolabeled panitumumab was confirmed by incubation of one set of cells with radiolabeled panitumumab with 10 µg unlabeled panitumumab. Assay results showed that only 3.5 ± 0.5% of radioactivity was bound, demonstrating that binding was specific (Table 7-15).

Table 7-15. Assay Results for Immunoreactivity and Specificity of ^{89}Zr-panitumumab

Batch Number MBR-^{89}Zr-PAN	Immunoreactivity* (%)	Specificity* (Absorbed Radioactivity after Blocking) (%)
04-17-12-001	65% ± 5	3.5 ± 0.5
04-18-12-001	68% ± 4	4.0 ± 0.8
04-25-12-001	66% ± 4	3.0 ± 0.5

*Not a release test.

7.12 Information to Support the Stability of the Drug Substance

^{89}Zr-panitumumab was made according to the procedures described in this IND application. The final drug product was left at 4°C for up to 72 hours. At periodic times over the 72 hours, the product was measured for radiochemical purity using analytical HPLC. In addition, the ^{89}Zr-panitumumab product has been examined for changes in UV absorbance of the product peak with time. There was no detectable breakdown of the product by UV over that time period. These results are summarized in the Table 7-16, below.

Expiration dating: The expiration time is 48 hours for the product stored at 4°C. The HPLC measurements shown in Table 7-16 suggest there is almost no or little loss of radiolabel over 72 hours at 4°C temperature but TLC measures after 48 hours show that the product starts losing the label slowly. Even after 72 hours, the purity (>90%) of the product by radio TLC is acceptable for human injection. The uncertainties given in Table 7-16 are the 1 standard deviation counting error for the propagated counts to provide an idea of the accuracy of the measurements. For the HPLC there were no other radioactive peaks detected but there could be an impurity that is not detectable. We are using 3 standard deviations of the background taken as peak areas (our software does not allow a ruler determination of peak to peak noise)

as the limit of detection. From the data below we feel we have good supporting data that the product is stable to 48 hours after synthesis.

Table 7-16. ^{89}Zr-panitumumab Stability Test Results

Batch MBR-^{89}Zr-PAN-	Time Post-Synthesis (hour)	% Pure by HPLC	% Pure by TLC
04-17-12-001	2.0	99 ± 0.7	99.6 ± 1.2
04-18-12-001	2.0	99 ± 0.9	99.8 ± 1.0
04-25-12-001	2.0	99 ± 0.4	99.0 ± 0.5
04-17-12-001	22.0	99 ± 0.8	98.5 ± 1.0
04-18-12-001	24.0	99 ± 0.9	99.0 ± 1.0
04-25-12-001	25.0	99 ± 1.0	97.0 ± 1.0
04-17-12-001	49.0	99 ± 0.7	97.5 ± 1.0
04-18-12-001	45.0	99 ± 0.7	98.5 ± 1.0
04-25-12-001	47.0	99 ± 0.5	97.0 ± 1.0
04-17-12-001	73.0	99 ± 0.8	96.5 ± 1.0
04-18-12-001	75.0	99 ± 0.7	97.0 ± 1.3
04-25-12-001	72.0	99 ± 0.6	96.0 ± 0.5

Serum Stability: This study was performed by HPLC analysis of the product (~50 µCi) incubated in 1.0 mL of whole human serum at 37°C. Over 5 days of analysis it was observed that the loss of labeling of 3 batches was 8 ± 2%. A representative diagram of the stability study for the product in human serum is shown, below, in Figure 7-16.

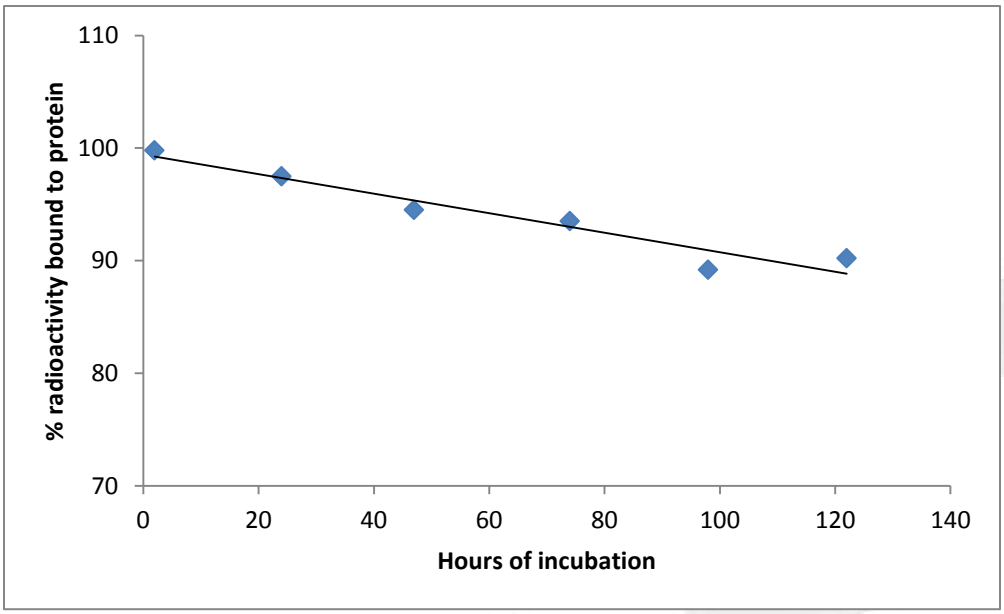
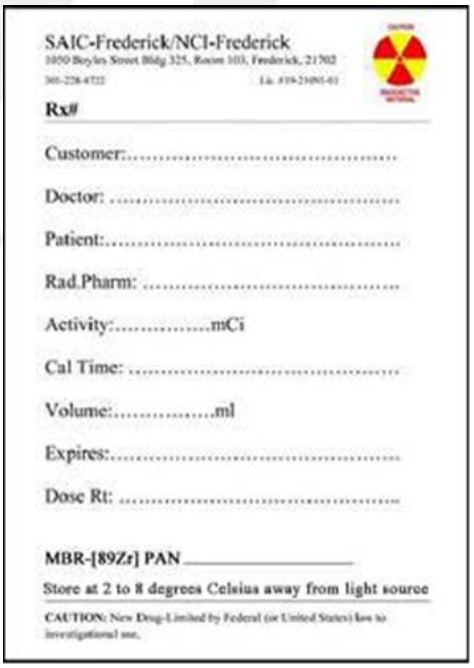


Figure 7-16. Stability of [89]Zr-panitumumab in Human Serum.
Approximately 50 µCi of the product was incubated in 1.0 mL of human serum at 37°C.

7.13 Drug Product Vial Labeling

An example label for drug product vialing is shown below:


SAIC-Frederick/NCI-Frederick

Rx#

Customer:.....

Doctor: .....

Patient:.....

Rad.Pharm: .....

Activity:.....mCi

Cal Time: .....

Volume:.....ml

Expires:.....

Dose Rt: .....

MBR-[89Zr] PAN _____

Store at 2 to 8 degrees Celsius away from light source

CAUTION: New Drug-Limited by Federal (or United States) law to investigational use.

7.14 Environmental Assessment

We request a categorical exclusion for the Environmental Impact Statement requirement in Section 21 CFR Part 25 because this radiopharmaceutical meets the conditions stipulated in Section 25.24(c)(4). Namely ^{89}Zr-panitumumab involves a long-lived radionuclide (T1/2 = 78.3 hours) prepared in unit dose and resulting in a total mass of active drug that does not exceed 1 mg. This procedure will be done no more than 100 times per year. Thus the amount of the waste that is expected to enter the environment may be reasonably expected to be nontoxic.

7.15 Supporting Information for CMC

The following items are included in this section as supporting material:

7.15.1 Completed Manufacturing Batch Record

7.15.2 Final Product QC Test Results and Updated Form V2

7.15.3 List of Approved SOPs for the Preparation of ^{89}Zr-panitumumab

7.15.4 Certificate of Analysis for DFO

7.15.1 Completed Manufacturing Batch Record

Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

^{89}Zr-labeled protein: Master Batch Record

Batch No.: MBR-^{89}Zr-PAN-041712-001 Manufacturing Date: 4/17/12 (Expires 48 hr EOS)

Equipment Description	Internal Tracking Number/ Identification	Manufacturer	Verified By (Initials)
HPLC System		Shimadzu	J.S
HPLC column		Superdex TM 200 10/300GL, GE Healthcare	J.S.
Dose Calibrator (calibrated ion chamber)		Capintec Inc.	J.S.
Thermomixer		VWR	J.S

GENERAL (Non-Inventoried per Batch) SUPPLIES	Verified By (Initials)
Appropriate Safety Wear including radiation badges, lab coat, safety glasses, shielding, gloves	J.S
2 beakers. One for filter integrity test, one for clean up.	J.S
Container for Solvent Waste, 500mL bottle	J.S

Reagents / Compounded Solutions	Internal Tracking Number/ Identification	Quantity	Verified By (Initials)
89Zr-Protein supplies kit	Form-S-89Zr-Protein-	1	J.S
89Zr-Protein Precursor, monoclonal antibody	SS-Protein-2012-01	8.0-10.0 mg	J.S.
Freshly prepared Gentisic acid solution (GenA-L-)	SS-GentA-2012-01	~30 mL	J.S.
89Zr-oxalate	SS-89Zr-Oxa-041712-01	~ 5 mCi	J.S.

Setting up of analytical HPLC system

a) HPLC system power on. Computer on and Windows software initialized.
b) Add ≥ 500 mL of mobile phase (**PBS-HPLC-L**) to eluent bottle 1
Mobile Phase: volume added: 1000 mL (≥ 500 mL)
c) Make certain that the Superdex HPLC column (**SS-Super**) is installed.
d) Run the method previously set for this analysis. Check that the flow is steady.
e) Make sure the column is clean and there is no unusual UV absorption (280 nm) until 30 min.
f) Go to the software menu bar and select manual stop. Reset the program.

JS initials.

89Zr-protein: 89Zr-protein Injection Master Batch Record Version: **V1**

Effective Date:_____ Supersedes:

Author:_____ **Date:** 04/16/2012
Signature

Regulatory Approval:_____ **Date:** **5/29/2012**
Signature

Procedure becomes effective on latest date of the two approval signatures above.
Procedure applies to 89Zr-protein IND.

DFO and PROTEIN CONJUGATION PROCEDURE

A. Protein Transfer

1. Check the protein concentration 20 mg/ml J.S. initials
2. Transfer the adequate volume (approximately 0.5 ml) of protein to a 1.5 mL microcentrifuge tube 0.5 ml J.S. initials
3. Calculate total mass of protein as Volume transferred x Concentration

   0.5 ml x 20 mg/ml = 10 mg J.S. initials

4. Recheck the calculations: J.S. initials

B. Calculation of SCN-Bz-DFO amount

1. Use 20 mM (15 mg/mL) stock solution (**DFO-L-**) of SCN-Bz-DFO in DMSO

2. Calculate the amount of DFO needed to label the antibody using the formula:

(Protein mass in mg)/MW of protein) * 3 *752.9 = DFO needed in mg

$$\frac{mg\ protein\ x\ 3\ x\ 753}{147000} = 0.14\ mg\ DFO$$

Protein mass in mg is from step **A** above
Protein MW is147000 g/mole (panitumumab)
SCN-Bz-DFO MW is 752.9 g/mole
SCN-Bz-DFO to protein molar mixing ratio is 3

Calculate the volume of DFO solution needed in µL as mg needed/15 mg/ml x 1000

$$\frac{mg\ DFO\ *\ 1000\ \mu L}{15\ mg} = 9\ \mu L\ of\ DFO$$

DFO needed: 0.14 mg J.S. initials

DFO needed: 9 uL J.S initials

Calculations checked by: J.S. initials

C. Conjugation of DFO to protein

1. Adjust pH of the antibody solution to pH= ~ 9.0 with 0.1M Na2CO3 (max. 0.1ml) using metal free pipette and pH paper (**SS-pH**).

2. Transfer the adequate volume (uL) of DFO solution (from step **B)** to the protein solution using metal free pipette to give a 3-fold molar excess of the DFO over the

molar amount of protein and mix immediately. Volume added: 9 uL ____
J.S. initials

3. Incubate the reaction for 1 hour at 37°C using a Thermomixer.

Time of incubation 60 min J.S. initials

D. DFO-protein Purification

1. Rinse/equilibrate a PD-10 column (**SS-PD10**) with 20ml 0.9% NaCl.
2. Pipette the conjugation reaction mixture onto the column and discard the flow-through.
3. Pipette 1.5ml 0.9% NaCl onto the column and discard the flow-through.
4. Pipette 2 ml 0.9% NaCl onto the PD-10 column and collect the DFO-protein in 0.5mL fractions. Combine fractions 2 to 4 to get DFO-conjugated protein in a 4mL glass vial.

E. Conjugated protein's concentration Determination and purity check by HPLC

1. Start HPLC using the method set-up in the beginning.
2. Inject 10 uL sample solution to the HPLC instrument.
3. The UV peak (280 nm) is analyzed according to the standard concentration curve of DFO conjugated protein (**SAIC-Frederick-Q119**) to get the concentration of the conjugate.

Calculated protein concentration: 5.2 mg/ml
Calculate the volume needed for 1 mg of protein:

$$\frac{1\ mg}{5.2\ \frac{mg}{mL}} = 0.2\ volume\ needed\ (ml)$$ J.S. initials

Verified by: J.S initials

^{89}Zr LABELING

F. Labeling procedure

1. Add 1M Oxalic Acid (**Oxa-L-**) to the 89Zr stock vial to make the specific concentration ~20 mCi/mL. Pipette the required volume (4 mCi, ~ 200 µL) of ^{89}Zr oxalic acid solution into a reaction vial.

Volume of 89Zr added 90 µL J.S. initials

2. Calibrate the reaction vial using dose calibrator (calibrated for 89Zr) to determine the amount of activity.

Amount of activity: 3.59 mCi J.S. initials

3. Pipette 90 µL 2M Na_2CO_3 (**NaCO-L-2-**) into the reaction vial to incubate for 3 minutes at room temperature. Adjust reaction solution pH to 7.5 using 2M Na_2CO_3.

4. While gently shaking:

   a) Pipette 0.5 ml 0.5M HEPES (pH7.2)

   b) Add the volume of the conjugate to get 1-1.2 mg mass of conjugate as calculated above.
   Volume added: 200 µL Conjugate mass 1 mg J.S. initials

   c) Add 0.2 mL 0.9% NaCl with Gentisic Acid (**GenA-L**)) into the reaction vial.

5. Incubate the mixture for 1 hour at room temperature.

G. Radiochemical yield of crude product using ITLC

1. Dilute approximately 2 µL of crude product to 1 mL with 0.9% NaCl.

2. Perform ITLC experiment following standard procedure (**SAIC-Frederick-Q322**).

3. If yield is < 50 % , reject the batch and start new labeling reaction

H. ^{89}Zr-DFO-Protein Purification

1. Rinse/equilibrate a PD-10 column (**SS-PD10**) with 20 mL of mobile phase (**GentA-L-**).

2. Pipette the conjugation reaction mixture onto the column and discard the flow-through.

3. Pipette 1.5 mL mobile phase onto the column and discard the flow-through.

4. Pipette 2 mL mobile phase onto the PD-10 column and collect the 89Zr-DFO-protein in 0.5 mL and 1.5 fractions in small glass vials. Check amount of activity each fraction in dose calibrator.

   1. 0.03 mCi;
   2. 2.68 mCi; J.S. initials

5. Pipette 0.5 mL mobile phase onto the PD-10 column and collect it as 3rd fraction. Check amount of activity in dose calibrator.

   3. 0 mCi J.S. initials

6. Check the radiochemical purity of fraction 2 and 3 by ITLC (**SAIC-Frederick-Q322**)
7. Add the fractions that are >95% pure to a 10 ml vial. Check the amount of radioactivity using dose calibrator.

Activity 2.68 mCi; time 14:25 A.M./P.M. J.S. initials

I. Sterile filtration of the final product

1. Dilute the product to 5 mL using 0.9% saline.
2. Draw the product solution in a 10 mL syringe
3. Remove the needle and attach sterile filter (**SS-SPF-protein**).
4. Connect filter needle to a 10 mL sterile vial with venting needle (**SS-VFN**).
5. Pass the solution through sterile filter slowly by pressing the plunger of the syringe.
6. Remove filter from vial and perform bubble point test of the sterile filter following standard procedure (**SAIC-Frederick-Q111**).

   Filter test result: 56 psig J.S. initials
7. Assay the product vial.

   Activity 2.49 mCi; time 14:35 A.M./P.M. J.S. initials
8. Draw ~ 200 µL of the product for QC tests.
9. Record the specific concentration of the product 0.5 mCi/mL

Operator signature _____ **Date** 4/17/12

Performed By: _____ **Date:** 4/17/12 **Checked By:** _____ **Date:** 04/17/12
Signature *Signature*

We certify that 89Zr-protein Production Batch **MBR-89Zr-PAN-**04712-001 was prepared according to the Manufacturing Instructions in the Manufacturing Production Record **MPR-89Zr-PAN**. Any additional manufacturing modifications have been captured along with the appropriate control information as outlined in **SAIC-Frederick-M120**, "Documentation of Manufacturing Variances, and attached as appropriate.

LA The QC release tests are complete and this batch of product has been preliminarily released according to **SAIC-Frederick-R309**, "QC Testing, Review, and Final Release for 89Zr-protein" for administration to human subjects. Final approval will be complete once the results of the Final product Sterility Testing have been met and reviewed as per **SAIC-Frederick-Q117A**.

N/A The QC release tests are complete and this batch of product will not be released, as it has failed to meet at least one or more of the specifications outlined in **SAIC-Frederick-R309**, "QC Testing, Review, and

Final Release for 89Zr-protein," and the OOS investigation as per **SAIC-Frederick-Q015** could not rule out the possibility of product quality issues.

Date of Batch Completion: 05/03/12

Manufacturing Operator: _____ **Date:** 4/17/12
Signature

Manufacturing Manager: _____ **Date:** 4/17/12
Signature

Date Final Sterility Results Received: 05/03/12

Final Sterility Results: ✓ **Pass** N/A **Fail**

Final Status of 89Zr-protein Production Batch **MBR-89Zr-PAN-** 04712 - 001

CWS Final approval and release has been granted.

N/A After careful review and consideration of the QC release testing and OOS investigation, this batch of product *cannot* be granted final approval and release; and therefore cannot be used for any further testing, if it passed all but the sterility tests previously. Notify all doctors of previously treated patients within 24 hours if the sterility test does not pass specification.

Manufacturing Manager: _____ **Date:** 05/03/12
Signature

QA/Regulatory Affairs: _____ **Date:** 05/03/12
Signature

7.15.2 Final Product QC Test Results and Updated Form V2

Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

[^{89}Zr]Protein FINAL PRODUCT QC
Test Results

Batch Number: MBR-[^{89}Zr]PAN-04172-001 **Manufacturing Date:** 4/17/12

Test Description	SOP Reference	Specification	Test Result(s)	Performed By/ Date	Supervisor Check By/ Date
Radiochemical ID & Purity (Radio-TLC)	SAIC-Frederick- Q322	Rf < 0.5 Purity > 90%	Rf = 0.48 Purity = 99.8 %	J.S. 4/17/12	CWA 4/17/12
Radionuclidic ID (Half-Life Test)	SAIC-Frederick- Q112	74-82 hours	76 hours	CWA 4/17/12	CWA 4/17/12
Bacterial Endotoxin (LAL)	SAIC-Frederick- Q114/Q114A	< 175 EU per dose	(Pass) Fail *(Circle One)*	CWA 4/17/12	CWA 4/17/12
pH	SAIC-Frederick- Q115	6-8	pH 7.0	J.S. 4/17/12	CWA 4/17/12
Chemical & Radiochemical Purity (HPLC)	SAIC-Frederick- Q321	Radiochemical Purity > 95% Chemical Purity: (by UV@ 280, or 254 nm) PAN < 1 mg / dose	Radiochemical Purity = 99.8 % PAN = 0.45 mg/dose	J.S. 4/17/12	CWA 4/17/12
Chemical Purity (Particulates)	SAIC-Frederick- Q121	Clear, Colorless, No particulates	(Pass) Fail *(Circle One)*	J.S. 4/17/12	CWA 4/17/12
Filter Integrity by Bubble Point	SAIC-Fredrick Q111	> 46 psi (Pall 4612)	(Pass) Fail *(Circle One)*	J.S. 4/17/12	CWA 4/17/12
Sterility*	SAIC-Frederick- Q117A/B	Negative/ No Growth	(Pass) Fail *(Circle One)*	CWA 05/03/12	CWA 05/03/12

[89Zr]Protein Final Product QC Test Results **Form FQC-006**
Version:V1 **Version Date:** _____
Reason for revision: Not Applicable **Original Date:** _____
Procedure becomes effective on latest date of 2 approval signatures Supersedes: **None**

Author: _____ **Date:** 4/16/2012

Regulatory Approval: _____ **Date:** 7/27/2012

Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

Test Not Required for Preliminary Release

Attach all test results to this QC Form, corresponding to the testing performed for the batch referenced above. Note any outstanding QC Investigations that are outstanding in the comment section below. For all doses, note the volume of each dose and ug in the dose in the comment section below.

Comments:

_____

_____

Preliminary Release By: _____ **Date:** 4/17/12
Signature

Final Release By: _____ **Date:** 5/03/12
Signature

Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

[^{89}Zr]Protein FINAL PRODUCT QC
Test Results

Batch Number: MBR-[^{89}Zr]PAN-_____ Manufacturing Date: _____

Test Description	SOP Reference	Specification	Test Result(s)	Performed By/ Date	Supervisor Check By/ Date
Radiochemical ID & Purity (Radio-TLC)	**SAIC-Frederick- Q322**	**Rf < 0.5 Purity > 90%**	**Rf = _____ Purity = _____%**		
Radionuclidic ID (Half-Life Test)	**SAIC-Frederick- Q112**	**74-82 hours**	**_____hours**		
Bacterial Endotoxin (LAL)	**SAIC-Frederick-Q114/Q114A**	**< 175 EU per dose**	**Pass Fail** (*Circle One*)		
pH	**SAIC-Frederick- Q115**	**6-8**	pH_____		
Chemical & Radiochemical Purity (HPLC)	**SAIC-Frederick- Q321**	**Radiochemical Purity > 95% Chemical Purity: (by UV@ 280, or 254 nm) PAN < 1 mg / dose**	**Radiochemical Purity = _____%** **PAN = _____mg/dose**		
Chemical Purity (Particulates)	**SAIC-Frederick- Q121**	**Clear, Colorless, No particulates**	**Pass Fail** (*Circle One*)		
Specific activity	**SAIC-Frederick- Q119**	**≥ 1.5 mCi/mg of mAb**	**Pass Fail** (*Circle One*)		
Filter Integrity by Bubble Point	**SAIC-Fredrick Q111**	**> 46 psi (Pall 4612)**	**Pass Fail** (*Circle One*)		

[89Zr]Protein Final Product QC Test Results **Form FQC-006**
Version:V2 **Version Date: _____**
Reason for revision: Not Applicable **Original Date: _____**
Procedure becomes effective on latest date of 2 approval signatures Supersedes: **V1**

Author:_____ Date:_____ .

Regulatory Approval:_____ Date: 9/5/2012

Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

Test Description	SOP Reference	Specification	Test Result(s)	Performed By/ Date	Supervisor Check By/ Date
Sterility*	**SAIC-Frederick-Q117A/B**	**Negative/ No Growth**	**Pass Fail** (*Circle One*)		

Test Not Required for Preliminary Release

Attach all test results to this QC Form, corresponding to the testing performed for the batch referenced above. Note any outstanding QC Investigations that are outstanding in the comment section below. For all doses, note the volume of each dose and ug in the dose in the comment section below.

Comments:

_____

_____

Preliminary Release By: _____ **Date:** _____
Signature

Final Release By: _____ **Date:** _____
Signature

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7.15.3 List of Approved SOPs for the Preparation of [89]Zr-panitumumab

"Insert Your Institution Information Here"

Document Control Number	Title (SOP)	Version No. & Effective Date
Oxa-L-	*Master Compounding Record Preparation of 1.0 M Oxalic acid for dilution of 89Zr-oxalate*	*V1*, 04/16/2012
DFO-L-	*20 mM desferrioxamine chelate solution in DMSO*	*V1*, 04/16/2012
NaCO-L-2-	*Master Compounding Record Preparation of 2M Na2CO3*	*V1*, 04/16/2012
NaCO-L-0.1-	*Master Compounding Record Preparation of 0.1 M Na2 CO3*	*V1*, 04/16/2012
GenA-L	*Master Compounding Record for Production of gentisic acid solution in saline*	*V1*, 04/16/2012
CHELEX-L-	*Master Compounding Preparation of Chelex-water*	*V1*, 04/16/2012
PBS-HPLC-L-	*Master Compounding Record for Preparation of Analytical HPLC Mobile Phase for 89Zr-Protein (PBS-NaN3-NaCl)*	*V1*, 04/16/2012
HEPES-L	*Master Compounding Record for Preparation of 0.5 M HEPES buffer*	*V1*, 04/16/2012
CITRICA-L	*Master Compounding Record for Preparation of TLC Mobile Phase for QC*	*V1*, 04/16/2012
MBR-[89Zr]protein	*Master Batch Record For Synthesis of 89Zr-protein Injection*	*V1*, 04/16/2012
MPR-[89Zr]protein	*Master Production Record For Synthesis of 89Zr-protein Injection*	*V1*, 04/16/2012
SAIC-Frederick-M001	*Standard Operating Procedure Writing & Revising Reagent & Supply Forms*	*V5, 06/18/2011*
SAIC-Frederick - M120	*Standard Operating Procedure Documentation of Manufacturing Variances*	*V4, 07/26/2011*
SAIC-Frederick - Q003	*Standard Operating Procedure Receiving, Handling, Quarantine, and Release of Reagents & Supplies*	*V4, 06/18/2011*
SAIC-Frederick - Q012	*Standard Operating Procedure Quality Systems Administration*	*V4, 06/18/2011*
SAIC-Frederick - Q015	*Standard Operating Procedure Investigating Out of Specification QC Test Results*	*V4, 07/19/2011*
SAIC-Frederick - Q108	*Standard Operating Procedure Resterilization of Final Product in the Event of a Failed Filter Integrity Test*	*V4, 06/18/2011*
SAIC-Frederick - Q111	*Standard Operating Procedure Filter Integrity Test by Bubble Point*	*V1*, 04/16/2012
SAIC-Frederick - Q112	*Standard Operating Procedure Radionuclide Identity by Half-Life Determination*	*V1*, 04/16/2012

Document Control Number	***Title (SOP) [continued]***	**Version No. & Effective date**
SAIC-Frederick - Q114A	*Standard Operating Procedure for Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test Using the PTS (Portable Test System)*	*V4, 06/01/2011*
SAIC-Frederick - Q115	*Standard Operating Procedure pH Testing of Final Product*	*V4, 06/17/2011*
SAIC-Frederick - Q116	*Standard Operating Procedure for Lowry Assay of DFO-protein.*	*V1*, 04/16/2012
SAIC-Frederick - Q117A	*Procedure for the Sterility Test*	*V1*, 04/16/2012
SAIC-Frederick - Q119	*Standard Operating Procedure for Determination of Concentration of DFO-protein by HPLC.*	*V1*, 04/16/2012
SAIC-Frederick - Q120	*Standard Operating Procedure for determination of number of DFO per protein molecule (89Zr-assay)*	*V1*, 04/16/2012
SAIC-Frederick - Q321	*Standard Operating Procedure of HPLC for 89Zr-protein*	*V1*, 04/16/2012
SAIC-Frederick - Q320	*Standard Operating Procedure of analytical HPLC for DFO-protein*	*V1*, 04/16/2012
SAIC-Frederick - Q322	*Standard Operating Procedure Radio-Thin Layer Chromatography Analysis for radiolabeled protein*	*V1*, 04/16/2012
SAIC-Frederick - R004	*Standard Operating Procedure Assigning Batch & Compounding Record Numbers to Production Runs*	*V4, 06/17/2011*
SAIC-Frederick - R005	*Standard Operating Procedure Drug Product Complaints*	*V4, 07/30/2011*
SAIC-Frederick - R006	*Standard Operating Procedure Product Labeling*	*V6, 06/18/2011*
SAIC-Frederick - R008	*Standard Operating Procedure Good Documentation Practices*	*V4, 06/17/2011*
SAIC-Frederick - R007	*Standard Operating Procedure: 89Zr-proteinQC Testing, Review*	*V1*, 04/16/2012
SAIC-Frederick - R009	*General Equipment Maintenance for 89Zr-protein Production*	*V1*, 04/16/2012

Document Control Number	***Title (Forms)***	**Version No. & Effective date**
Form S-89Zr-protein	*89Zr-protein Supplies (Kit)Preparation Form*	*V1*, 04/16/2012
FQC-006	*89Zr-protein Final Product QC Test Result*	*V2*, 09/5/2012
Form R004-PBS-HPLC-L	*Compounding Record Number Log for Analytical HPLC Mobile Phase for 89Zr-protein*	*V1*, 04/16/2012
Form R004-ProteinSTD-L	*Compounding Record Number Log for Preparation of HPLC Standards for 89Zr-protein and DFO-protein*	*V1*, 04/16/2012
Form R004-0.1 M Na2CO3	*Compounding Record Number Log for Prep of 0.1 M Na2CO3 solution*	*V1*, 04/16/2012
Form R004-2 M Na2CO3	*Compounding Record Number Log for Prep of 2 M Na2CO3 solution*	*V1*, 04/16/2012
Form R004-0.5 M HEPES	*Compounding Record Number Log for Prep of 0.5 M HEPES buffer*	*V1*, 04/16/2012
Form R004-HPLC-L	*Compounding Record No. Log for Prep. of Analytical HPLC Mobile Phase for 89Zr-protein*	*V1*, 04/16/2012
Form R004-MBR: 89Zr-protein	*Master Batch Record Number Log for Synthesis of 89Zr-protein*	*V1*, 04/16/2012
From R004-S-89Zr-protein-L	*89Zr-protein Kit Preparation Record Log*	*V1*, 04/16/2012
Form R004-GENTA	*Compounding Record Number Log for Prep of Gentisic acid acid*	*V1*, 04/16/2012
Form R004-Citric	*Compounding Record Number Log for Prep of citric acid*	*V1*, 04/16/2012
Form R004-1.0 M OXA	*Compounding Record Number Log for Prep of 1.0 M oxalic acid*	*V1*, 04/16/2012
Form R004-DFO-L-	*Compounding Record Number Log for Prep of 20 mM DFO in DMSO*	*V1*, 04/16/2012

Document Control Number	*Reagent/Supply*	Version No. & Effective date
SS-DFO	*Desferrioxamine chelate reagent /Supply Specification Sheet*	*V1*, 04/16/2012
SR-DFO	*Desferrioxamine chelate Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-CHELEX	*Chelex-resin reagent /Supply Specification Sheet*	*V1*, 04/16/2012
SR-CHELEX	*Chelex-resin Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-Super	*GE Healthcare Superdex TM 200 analytical column for Zr-protein Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-Super	*Superdex TM 200 analytical column Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-SOC	*Sodium Carbonate Reagent/Supply specification Sheet*	*V1*, 04/16/2012
SR-SOC	*Sodium Carbonate Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-Pani	*Protein Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-Pani	*Protein Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-Zr-Cl	*Cold Zr-chloride Reagent/supply specification sheet*	*V1*, 04/16/2012
SR-Zr-Cl	*Cold Zr-chloride Reagent shipping receipt and verification*	*V1*, 04/16/2012
SS-89Zr-OXA	*89Zr-oxalate reagent/supply specification sheet*	*V1*, 04/16/2012
SR-89Zr-OXA	*89Zr-oxalate reagent and receipt verification*	*V1*, 04/16/2012
SS-DMSO	*DMSO Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-DMSO	*DMSO Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-NSI	*0.9% Sodium Chloride, injection, USP Reagent/Supply Specification Sheet*	*V3, 06/18/2011*
SR-NSI	*0.9% Sodium Chloride Shipping Receipt and Verification*	*V3, 06/18/2011*
SS-OXA	*Oxalic Acid Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-OXA	*Oxalic Acid Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-WFI	*Sterile Water for Injection Reagent/Supply Specification Sheet*	*V2, 06/18/2011*
SR-WFI	*Sterile Water for Injection, USP Shipping Receipt and Verification*	*V2, 06/18/2011*
SS-WIR	*Sterile Water for Irrigation, USP Reagent/Supply Specification Sheet*	*V2, 07/19/2011*
SR-WIR	*Sterile Water for Irrigation, USP Shipping Receipt and Verification*	*V2, 07/19/2011*
SS-PD10	*GE Healthcare size exclusion PD10 column Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-PD10	*PD10 column Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-GentA	*Gentisic acid Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-GentA	*Gentisic acid Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-CitricA	*Citric acid Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-CitricA	*Citric acid Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-NaN3	*NaN3 Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-NaN3	*NaN3 Shipping Receipt and Verification*	*V1*, 04/16/2012
SS-PBS10M	*PBS 10 M buffer Reagent/supply specification sheet*	*V1*, 04/16/2012
SR-PBS10M	*PBS 10 M buffer shipping receipt verification*	*V1*, 04/16/2012

Document Control Number	***Reagent/Supply [continued]***	**Version No. & Effective date**
SR-ITLC	*ITLC plates shipping receipt verification*	*V1*, 04/16/2012
SS-pH	*pH strip Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SS-HGW	*HPLC Grade Water Reagent/Supply Specification Sheet*	*V1*, 06/18/2011
SR-HGW	*HPLC grade water Shipping Receipt and Verifications*	*V1*, 06/18/2011
SS-SBV	*Sterile Borosilicate Vials Reagent/Supply Specification Sheet*	*V4,* 07/22/2011
SR-SBV	*Sterile Borosilicate Vials Shipping Receipt and Verifications*	*V4,* 07/22/2011
SS-VFN	*Venting Filter Needle Reagent/Supply Specification Sheet*	*V3,* 07/22/2011
SR-VFN	*Venting Filter Needle Shipping Receipt and Verification*	*V3,* 07/22/2011
SS-SPF-protein	*Sterile Product Filter Reagent/Supply Specification Sheet*	*V1*, 04/16/2012
SR-SPF-protein	*Sterile Product Filter Shipping Receipt and Verification*	*V1*, 04/16/2012

7.15.4 Certificate of Analysis for DFO

Macrocyclics Certificate of Analysis

General Product Data

Product: *p*-SCN-Deferroxamine

Scientific Name: 1-(4-Isothiocyanatophenyl)-3-[6,17-dihydroxy-7,10,18,21-tetraoxo-27-(N-acetylhydroxylamino)-6,11,17,22-tetraazaheptaeicosane]thiourea

Catalog Number: B-705

CAS Number: N/A

Theoretical Formula: $C_{33}H_{52}N_8O_8S_2$

Theoretical Formula Weight: 752.9 g/mol

Lot Specific Data

Macrocyclics Reference Number: B705PD10003-091111a

Characteristic	Test Method	Acceptance Criteria	Result	Pass/Fail
Identity	FTIR	Conforms to reference	Conforms	Pass
Chromatographic Purity at 275nm	HPLC	≥ 94% (area %)	93.9%	Pass

Quality Manager: 04-30-10

7.16 References

A list of references for Item 7 is provided below:

17. Vosjan MJWD, Perk LR, Visser GWM, Budde M, et al. Conjugation and radiolabeling of monoclonal antibodies with zirconium-89 for PET imaging using the bifunctional chelate *p* isothiocyanatobenzyldesferrioxamine. Nature Protocols, 2010;4:739.

18. Verel I, Visser GWM, Boellaard R, Sigter-van Walsum M, Snow GB, et al. 89Zr-Immuno-PET: comprehensive procedures for the production of 89Zr-labeled monoclonal antibodies. J Nucl Med, 2003;44:1271-81.

19. Liu S, Edward DS. Stabilization of (90)Y-labeled DOTA-biomolecule conjugates using gentisic acid and ascorbic acid. Bioconj Chem, 2001;12(4):554-8.

20. Bhattacharyya S, Wei L, Riffle L, Hill G, et al. Preclinical evaluation of 89Zr-labeled panitumumab as a potential PET probe for HER1-expressing carcinomas. J Nucl Med Meeting Abstracts, 2012;53 (Supplement 1):1693.

21. Szajek LP, Meyer W, Plascjak P, Herscovitch P. Expanding the utility of a GE PETtrace cyclotron. J Labelled Comp, 2007;50:S93.

22. Holland JP, Shey Y, Lewis JS. Standardized methods for the production of high specific-activity zirconium-89. Nucl Med Biol, 2009;36:729-39.

23. FDA Guidance for Industry: ICH Q3C–Tables and List, Revision 2 (2/2012). http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073395.pdf. (Last Accessed August 13, 2012)

24. Nayak TK, Garmestani K, Milenic DE, Brechbiel MW. PET and MRI of Metastatic Peritoneal and Pulmonary Colorectal Cancer in Mice with Human Epidermal Growth Factor Receptor 1-Targeted 89Zr-Labeled Panitumumab. J Nucl Med, 2012;53(1):113-20.

Clinical Hold Complete Response

Response to Clinical Hold

for

^{89}Zr-panitumumab IND

IND Sponsor:

Insert Your Institution Information Here

The FDA's requests in the Full Clinical Hold Letter dated September 19, 2012 are provided below in bold, italic type immediately followed by [Insert your instituion name here] response.

Clinical hold issue:

"You have not provided complete information for the isotope. Submit a certificate of analysis (CoA) for the isotope along with either additional isotope manufacturing information or letters of cross-reference to the manufacturing information. It is recommended that the CoA include radiochemical purity and impurities, radionuclide purity and impurities, specific activities, and energy sources. In addition, the sterility and endotoxin free nature of each isotope should be documented as described in the 1997 FDA Guidance "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/OtherRecommendationsforManufacturers/UCM153182.pdf)."

Response:

We will re-sterilize the isotope in the pharmacy and perform appropriate quality assurance testing (see table below) including filter bubble testing, endotoxin and sterility per established SOPS, and document the result on Form FQC-007. Additional requirements for the drug product made and tested on the same day are documented in FQC-006.

A certificate of analysis is not appropriate. We believe the agency has misunderstood the source of the isotope and/or the receiving entity. The isotope [^{89}Zr]oxalate is not purchased from an outside vendor. Certificates of analysis are issued by vendors when they sell materials to a customer, they are not used for materials produced for internal use that are processed immediately and used in an integrated process within the same institution. [Insert your institution/radiopharmacy name here] is an [insert your institution/radiopharmacy name here] facility, as is the cyclotron facility. We may have caused this misunderstanding in our original application, when we referred to the [insert your cyclotron or vendor name here] and to [Insert your institution/radiopharmacy name here], perhaps leading you to conclude incorrectly that the radiopharmacy was not an [insert your institution name here] facility. We have attached a document describing [insert your institution name here] and the subgroups involved in this IND – all are part of [insert your institution name here] . In-process testing is the appropriate procedure in this situation and we will document in-process testing in Form FQC-007. [^{89}Zr]oxalate is produced at [insert your cyclotron or vendor name here] in the cyclotron facility only when specifically requested. Following processing in 6N HCl and H_2O_2 at 90°C and purification into 1.0 M oxalic acid (pH<1), the isotope is transferred the same day to the nuclear pharmacy at [insert your cyclotron or vendor name here] , where is it immediately used to label the protein. We do not believe that additional sterilization of the isotope solution is needed. It adds no additional assurance of sterility, given the aggressive processing and formulation and exposes our staff to additional radiation. However, we have added a process to re-sterilize the isotope solution as that appears to be a non-negotiable condition to activating this IND.

A typical batch record with the manufacturing conditions for the cyclotron production of the isotope is attached as well as a cyclotron quality control result sheet with the activity and the gamma spectrum data, identifying the isotope and the presence or absence of isotopic impurities. The specific concentration of ^{89}Zr and % of ^{88}Zr impurity must be in the acceptable range and are documented in Form FQC-007.

Note that all processes to prepare and test the final clinical dose are completed within hours of the final isotope processing at the cyclotron. Additional QC in-process tests will be performed in the pharmacy before releasing this material for radiolabeling and final clinical doses will not be released until the in process QC is approved.

All radioisotope solution manipulation is performed inside the lead shielded ISO class-5 hood. The solution is first diluted to 1-2 mL with 1.0 M oxalic acid (Oxa-L-). Then, using a 5.0 mL sterile shielded syringe, all of the sample is passed through a 0.22 micron sterile filter (SS-SPF) into a sealed sterile vial vented with a sterile venting needle (SS-VFN). The filter integrity test is performed following standard SOP (Q111). Sterile filtered isotope solution will be visually inspected for its clarity and colorlessness (Q121). Approximately 10 to 20 µL of the filtered solution will be withdrawn for bacterial endotoxin (Q114A) and sterility (Q117A) tests. When all these test results satisfy the acceptance criteria (as stated in FQC-007, the sterility results will not be available until later) the isotope is acceptable to produce human doses. FQC-007 form will be completed and signed before the final radiopharmaceutical can be used in the clinical imaging suite for administration to human subjects. The final radiopharmaceutical QC form (FQC-006) has been updated to reflect the satisfactory completion of the isotope quality testing. Since specific activity and isotope half-life are determined on the final radiopharmaceutical on the same day as the isotope is processed it is not necessary to duplicate these tests on the isotope.

Specifications:

The acceptance criteria for the ^{89}Zr-oxalate solution are shown in Table 1 and for the final radiopharmaceutical in Table 2:

Table 1. ^{89}Zr Oxalate In-Process Specifications (FQC-007)

Test Description	SOP Reference	Specification
Specific concentration*	QC sheet with ^{89}Zr-oxalate	≥ 15 mCi/mL
Zr-88 impurity*	QC sheet with ^{89}Zr-oxalate	≤ 0.4 %
Bacterial Endotoxin (LAL)	Q114/Q114A	< 175 EU per dose
Chemical Purity (Particulates)	Q121	Clear, Colorless, No particulates
Filter Integrity by Bubble Point	Q111	≥ 45 psi
Sterility**	Q117A/B	Negative/No Growth

**Test will be performed at the cyclotron facility, Bethesda. Results will be provided with ^{89}Zr-oxalate.*

*** **Test Not Required for Preliminary Release***

Table 2. [89]Zr-panitumumab Specifications (FQC-006)

Description	SOP Reference	Specification
[89]Zr-oxalate COA	FQC-007	Passes all requirement
Radiochemical ID & Purity (Radio-TLC)	Q322	Rf < 0.5 Purity > 90%
Radionuclidic ID (Half-Life Test)	Q112	74-82 hours
Bacterial Endotoxin (LAL)	Q114/Q114A	< 175 EU per dose
pH	Q115	6-8
Chemical & Radiochemical Purity (HPLC)	Q321	Radiochemical Purity > 95% Chemical Purity: (by UV@ 280, or 254 nm) PAN < 1 mg / dose
Chemical Purity (Particulates)	Q121	Clear, Colorless, No particulates
Specific activity	Q119	≥ 1.5 mCi/mg of mAb
Filter Integrity by Bubble Point	Q111	> 46 psi (Pall 4612)
Sterility*	Q117A/B	Negative/No Growth

****Test Not Required for Preliminary Release***

Testing Methods for [89]Zr-oxalate:

Specific Concentration. Activity is determined in a Capintec well counter at a setting of 465 and divided by the volume to obtain mCi/mL as supplied.

Gamma spectroscopy analysis. Radionuclidic purity is evaluated with a coaxial intrinsic germanium detector (Princeton gamma-tech, Inc.) coupled through a spectroscopy amplifier and multichannel buffer. The system is calibrated for energy and efficiency with mixed-radionuclide point sources standards (Analytics, Inc.) from 70 to 1836 keV. Analysis library data was obtained from Nuclide Navigator (EG&G Ortec). The γ-ray spectroscopy sample (100 µL) is drawn from the [Zr-89]Zr-oxalate stock solution, diluted and positioned (240 cm) above the surface of the detector yielding nearly point source geometry. Counting dead time is kept below 10%. HPGe γ-ray analysis of a [Zr-89]Zr-oxalate solution after processing indicate only the presence of Zr-89 (with Zr-88 as minor impurity)**.** The gamma spectrum is measured for a minimum of 2 hours. The percentage of Zr-88, Zr-89, Y-86, Y-87, and Y-89 are determined and reported. The percent of Zr-88 cannot exceed 0.4%. Y-86, Y-87, and Y-89 are below the minimum detectable amount (MDA).

Particulates. The [89]Zr- oxalate solution is examined visually. The chemical purity by visual inspection is straightforward, the material in the vial should be clear and colorless without any visible particulates as per USP <823> and USP <631> Color and Achromicity. The product must pass this test in order to be released.

Filter Integrity. Because the USP sterility test requires 14 days to complete, the ^{89}Zr-oxalate product solution sterility cannot be assured prior to use. In addition to working in an ISO class 5 environment, the ^{89}Zr-oxalate is passed through a 0.2 μm sterilizing filter into a sealed sterile vial vented with a sterile venting needle. After the ^{89}Zr-oxalate is collected, the sterilizing filter is tested for filter integrity to give an indication of likelihood of product sterility. Filter integrity is tested in a bubble point procedure, whereby the sterilizing filter is placed on a gas line with a pressure gauge and the outlet of the filter is placed under water. The gas pressure on the inlet to the filter is increased slowly until a steady stream of bubbles is observed at the filter outlet. The pressure when the bubble stream begins is recorded and compared with the manufacturer's pressure rating for the filter, from the certificate of quality. If the observed bubble point pressure exceeds the manufacturer's specifications, the filter integrity test is passed. If the bubble point pressure is too low, the filter may be rewetted and tested again. If the bubble point pressure is greater than the manufacturer's specifications, the test is passed; if not then the product must be resterilized by passing through a second sterilizing filter. That filter must then pass the bubble point test. The ^{89}Zr-oxalate may only be resterilized once. This test must be passed for the ^{89}Zr-oxalate to be released for use.

Bacterial Endotoxin. Levels are tested and qualified using one of two procedural methods; both are based on the USP recommendations, but using control standard endotoxin referenced to USP Reference Standard Endotoxin (RSE). Either a gel clot method is used with single test vials or the portable test system from Charles River Laboratories.

Sterility. Sterility is tested using the direct inoculation method as is required by the USP. The test is completed after release of the product.

APPENDICES:

[Insert your institution or radiopharmacy name here]	0007
^{89}Zr Processing Overview	0009
Batch Record for ^{89}Zr Production	0018
QC Form from Cyclotron Facility	0025
Form FQC-007	0027
Form FQC-006	0030
Updated List of Standard Operating Procedures	0033
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