Template

, QVHLW* RXU, QVWXXWRQ, QIRUP DWRQ + HLH

Not Responsible for Investigator's Use

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

Batch No.: MBR-^{89}Zr-PAN-_____ Manufacturing Date: _____ (Expires 48 hr EOS)

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

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

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

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: _____ 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.

_____ initials.

89Zr-protein: 89Zr-protein Injection Master Batch Record Version: **V1**
Effective Date:_____ Supersedes:

Author:_____**Date:**_____ .
Signature

Regulatory Approval:_____**Date:** _____ .
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 _____ mg/ml _____ initials
2. Transfer the adequate volume (approximately 0.5 ml) of protein to a 1.5 mL microcentrifuge tube _____ml _____ initials
3. Calculate total mass of protein as Volume transferred x Concentration

   _____ ml x _____ mg/ml =_____ mg _____ initials

4. Recheck the calculations: _____ 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

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

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

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

DFO needed: _____ mg _____ initials

DFO needed:_____ uL _____ initials

Calculations checked by: _____ 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

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molar amount of protein and mix immediately. Volume added: _____uL ____ _____initials

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

   Time of incubation _____min _____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 (**Form-Q119**) to get the concentration of the conjugate.

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

$$\frac{1\ mg}{_____\ \frac{mg}{mL}} = _____ volume\ needed\ (ml)$$ _____ initials

Verified by: _____ 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 _____µL _____initials

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

Amount of activity: _____mCi _____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:_____µL Conjugate mass _____mg _____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 (**Form-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. _____ mCi;
   2. _____mCi; _____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._____mCi _____initials

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

Activity _____mCi; time _____A.M./P.M. _____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 (**Form-Q111**).

   Filter test result: _____psig _____initials

7. Assay the product vial.

   Activity _____mCi; time _____A.M./P.M. _____initials

8. Draw ~ 200 µL of the product for QC tests.
9. Record the specific concentration of the product _____mCi/mL

Operator signature _____ Date

Performed By: _____ **Date:** _____ **Checked By:** _____**Date:**_____
Signature *Signature*

We certify that 89Zr-protein Production Batch **MBR-89Zr-PAN-_____** 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 **Form -M120**, "Documentation of Manufacturing Variances, and attached as appropriate.

_____ The QC release tests are complete and this batch of product has been preliminarily released according to **Form-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 **Form-Q117A**.

_____ 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 **Form-R309**, "QC Testing, Review, and

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

Date of Batch Completion: _____

Manufacturing Operator: _____ **Date:** _____
Signature

Manufacturing Manager: _____ **Date:** _____
Signature

Date Final Sterility Results Received: _____

Final Sterility Results: _____ **Pass** _____ **Fail**

Final Status of 89Zr-protein Production Batch **MBR-89Zr-PAN-_____**:

_____ Final approval and release has been granted.

_____ 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:** _____
Signature

QA/Regulatory Affairs: _____ **Date:**_____
Signature

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