Template

"Insert Your Institution Information Here"

Not Responsible for Investigator's Use

Master Production Record

MPR-[^{89}Zr]Protein

For Synthesis of [^{89}Zr] Protein: [^{89}Zr] Protein Injection

Purpose: This is the Master Production Record for synthesizing ^{89}Zr-protein for investigational use only.

REFERENCES & MATERIALS OVERVIEW

A. REFERENCES

1. **MBR-[^{89}Zr]Protein**, "[^{89}Zr]-Protein: [^{89}Zr]-Panitumumab Injection Master Batch Record "
2. **Form-Q121,** "Chemical Purity by Visual Inspection**"**
3. **Form-Q322,** "Radiochemical Identification & Purity of [^{89}Zr]-Protein Injection by Radio-Thin Layer Chromatography Analysis"
4. **Form-Q112**, "Radionuclidic Identification by Half-Life Determination"
5. **Form-Q114A,** "Alternative procedure for Bacterial Endotoxins by Limulus amoebocyte Lysate (LAL) Test Using the PTS (Portable Test System)"
6. **Form -Q115**, "pH Testing of Final Product"
7. **Form -Q117A**, " Alternative Procedure for the Sterility Test"
8. **Form -Q111**, "Filter Integrity Test by Bubble Point"
9. **Form -Q321**, "Chemical and Radiochemical Purity and Identity by HPLC for ^{89}Zr-protein"
10. **Form-Q320**, Standard Operating Procedure of analytical HPLC for DFO-protein
11. **Form -Q012**, "Quality Systems Administration"
12. **Form -Q015**, "Investigating Out of Specification QC Test Results"
13. **Form -R004**, "Assigning Batch & Compounding Record Numbers to Production Runs"
14. **Form -R005**, "Drug Product Complaints"
15. **Form -R006**, "Product Labeling"
16. **Form -R008**, "Good Documentation Practices"
17. **Form –R007**, "QC Testing, Review, & Final Release for 89Zr-protein"
18. **Form –R009**, "General Equipment Maintenance"
19. **Form FQC-006**, "[89Zr]Protein Final Product QC Test Results" Form
20. **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"

89Zr-protein: 89Zr-protein Injection Master Production Record Version: **V1**
Effective Date:_____ Supersedes: none

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.

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21. Form MVR-001, "Manufacturing Variance Report Form"
22. Form EQ-001, "General Equipment History Log"
23. Form R004-L, "General Log for Assigning Unique Batch or Compounding Record Numbers"
24. DFO-L, 20 mM desferrioxamine chelate solution in DMSO
25. NaCO-L-2, Master Compounding Record Preparation of 2M Na_2CO_3
26. **NaCO-L**, 0.1- Master Compounding Record Preparation of 0.1 M Na_2CO_3
27. **GenA-L**, Master Compounding Record for Production of gentisic acid solution in saline
28. CHELEX-L, Master Compounding Preparation of Chelex-water

B. EQUIPMENT

Equipment Description	Manufacturer
HPLC System	Shimadzu
HPLC column	**Superdex TM 200 10/300GL, GE Healthcare**
Dose Calibrator (calibrated ion chamber)	Capintec Inc.
Thermomixer	VWR

C. REAGENTS/MATERIALS

GENERAL (Non-Inventoried per Batch) SUPPLIES
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 Liquid Waste, 500mL bottle

Reagents / Compounded Solutions	Internal Tracking Number/ Identification	Quantity
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

D. SPECIAL NOTES AND POLICIES

• 89Zr emits very high energy gamma photons. Adequate shielding is essential to reduce the exposure during manipulation.
• Always use metal-free sterile pipette tips while transferring reagents and solvents.
• Always use chelex water.
• As 89Zr is an unusual radio-isotope, proper calibration of dose calibrator (ion chamber) is essential.

E. RESPONSIBILITIES

• Each site should ensure that their operators have all of the required credentials and prerequisite training required to synthesize radiopharmaceuticals. Document all manufacturing control information on the corresponding Master Batch Record, **MBR-[89Zr]-PAN-**.

F. SETTING UP AND SUITABILITY CHECK OF ANALYTICAL HPLC SYSTEM

i) Turn on the HPLC system and initialize the software
ii) Add ≥ 500 mL of mobile phase (**PBS-HPLC-L)** to eluent bottle 1 - Mobile Phase.
iii) Make certain that the Superdex HPLC column (**SS-Super**) is installed.
iv) Run the method previously set for this analysis. Check that the flow is steady.
v) Make sure the column is clean and there is no unusual UV absorption (280 nm) until 30 min.
vi) Go to the software menu bar and select manual stop. Reset the program.
vii) Check suitability of the HPLC by injecting standard protein solution () RUP R009,) RUP -Q320).
viii) Repeat step v and vi before starting the synthesis.

G. MANUFACTURING OF 89Zr-PROTEIN

a. DFO and PROTEIN CONJUGATION PROCEDURE

I. Protein Transfer

1. Check the protein concentration written in the vial
2. Transfer about 0.5 mL, measured accurately, of protein to a 1.5 mL microcentrifuge tube using metal free sterile micropipette tip.
3. Calculate total mass of protein transferred.
4. Recheck the Calculation.

II. 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 mass of DFO and the volume of the stock solution needed to label the antibody using the formula:

DFO needed in mg = (protein mass in mg/MW) * 3 *752.9 =
Volume of DFO solution = 1000 µL/mL X mg needed/15 mg DFO/ml

Protein mass in mg is from step **I** above
Protein MW is 147000 g/mole
SCN-Bz-DFO MW is 752.9 g/mole
SCN-Bz-DFO to protein molar mixing ratio is 3

Check the calculation twice.

III. 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 volume (µL) of DFO solution (from step **II)** 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.

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3. Incubate the reaction for 1 hour at 37°C using a Thermomixer. Note the time of incubation.

IV. DFO-protein Purification

1. Rinse/equilibrate a PD-10 column (**SS-PD10**) with 20 ml 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 2ml 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 4 mL glass vial.

V. Conjugated protein's concentration determination and purity check by HPLC

1. Start HPLC using method set up at the start of this procedure.
2. Dilute the sample 500 x by adding 20 µL of product to 0.5 mL of saline (SS-NSI)
3. Inject 10 uL diluted sample solution to the HPLC instrument
4. Integrate the HPLC chromatogram to get the UV peak (280nm) area
5. Calculated protein concentration (mg/ml) using standard calibration curve () RUP Q119).
6. Calculate the volume needed for 1 mg protein.

^{89}Zr-LABELING

VI. 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.
2. Calibrate the reaction vial using dose calibrator (calibrated for 89Zr) to determine the amount of activity in mCi.
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:
5. Pipette 0.5 ml 0.5M HEPES (pH7.2)

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a. b) Add adequate volume of the conjugate to get 1-1.2 mg mass of conjugate. Calculate based on step 18.

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

6. Incubate the reaction for 1 hour at room temperature.

VII. Radiochemical yield of crude product using ITLC

1. Dilute approximately 2 µL of crude product to 500 times with 0.9% NaCl.
2. Perform ITLC experiment following standard procedure (**Form-Q322**).
3. If yield is < 50 % , discard the whole batch and start new labeling reaction

VIII. ^{89}Zr-DFO-Antibody 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.
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.
6. Check the radiochemical purity of fraction 2 and 3 by ITLC (**Form-Q322)**
7. Combine fractions 2 to 3 if they are >95% pure in a 10 ml vial. Check the amount of radioactivity using dose calibrator.

IX. 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**).

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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. Perform bubble point test of the sterile filter following standard procedure (**Form -Q111**).

7. Detach the product vial from syringe attached with sterile filter.

C. POST SYNTHESIS

1. Assay the collection vial using the calibrated ion chamber. Record the assay time as the End of Synthesis Time in the corresponding batch record for this batch of product. Place the collection vial into the dose-drawing shield.

2. Visually inspect the product 89Zr-protein as per) RUP **-Q121** looking for particulates, describe clarity and color. If the sample is not free of particulates and colorless, the product cannot be used. Document the color and particulate state, i.e. free of particulates or not, on **Form FQC-006**.

3. Detach the sterilizing filter from the syringe, and place it in a lead shield for the filter integrity test. Perform the integrity test as per SOP **Form - Q110** and record the bubble point pressure on the batch record.

4. Swirl the vial to completely mix the contents. Swab the product [^{89}Zr]-Protein vial with an alcohol swab. Using a 0.5 mL tuberculin syringe, aseptically withdraw an accurate ~0.2 mL sample for Quality Control Testing, record the volume withdrawn. Replace the sheath on the needle. Assay the sample and then place the syringe in a lead shield. Re-swab the product vial. Transfer the shielded syringe to Quality Control for testing as summarized on **Form FQC-006**.

5. In the batch record, calculate the specific concentration in mCi/mL, at the End of Synthesis (EOS).

6. Aseptically use a (~ 22G) x 1½" needle on a 1 (or 3) mL syringe to withdraw a < 0.20 mL sample into the syringe for sterility tests (0.20 mL straight or 0.1mL can be used with an additional 0.5 mL of water for injection) and reserve sample (< 0.2 mL) and place the syringe in a lead shield. Transfer the shielded syringe to Quality Control for preparing the sterility samples and saving the reserve.

NOTE: The sterility sample must be inoculated within 48 hours of EOS. Remove and discard the vent filters.

7. Record the results of the QC release tests on **Form FQC-006**. The tests to be conducted should include the following:

• "Radiochemical Identification & Purity of ^{89}Zr-Protein Injection by Radio-Thin Layer Chromatography Analysis and HPLC co-injection," per SOP Form **-Q322 and Form Q321**
• "Radionuclidic Identification by Half-Life Determination," per SOP Form **-Q112**
• "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test," per SOP Form **-Q114 or** "Alternative Procedure for

Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test Using the PTS (Portable Test System), " per SOP **Form -Q114A.**
• "pH Testing of Final Product," per SOP **Form -Q115**
• "Chemical and Radiochemical Purity by HPLC for 89Zr-protein," per SOP **Form -Q322**
• "Chemical Purity by Visual Inspection," per SOP Form **-Q121**

8. Record any manufacturing variances as per SOP **Form -M120**, "Documentation of Manufacturing Variances," using **Form MVR-001**, and attach them to the corresponding batch record for this batch of product. Record the number of variances reported for this batch.
9. Attach appropriate labels to the 89Zr-protein product vial and in the batch production record where indicated.
10. Perform final review and release of QC test results as per Form **-R209**, "QC Testing, Review, & Final Release for 89Zr-protein."
11. Record final sterility results on **Form FQC-006** once they are complete and available. The sterility testing should be completed as per "Sterility Test," per SOP **Form -Q117 or** "Alternative Procedure for the Sterility Test," as per SOP **Form -Q117A.**

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