Template Provided By:

Cancer Imaging Program

National Cancer Institute

Master Production Record

GEN-MPR-[^{18}F]FES

For Synthesis of [^{18}F]-Fluoroestradiol: [^{18}F]FES Injection

Purpose: This is the Master Production Record for synthesizing [^{18}F]-Fluoroestradiol using the GE TRACERLab FX_{F-N}, an automated radiochemistry synthesis system, to produce this fluorine-18 labeled radiopharmaceutical product for investigational use only.

REFERENCES & MATERIALS OVERVIEW

A. REFERENCES

1. **MBR-[^{18}F]FES-L**, "[^{18}F]-Fluoroestradiol: [^{18}F]FES Injection Master Batch Record "
2. **GEN-Q121,** "Chemical Purity by Visual Inspection**"**
3. **TRACERLab FX $_{F-N}$ Operator Guide,** Direction 2368510-100, Rev 1
4. **TRACERLab FX Software Guide**
5. **GEN-Q311,** "Radiochemical Identification & Purity of [^{18}F]-Fluoroestradiol ([^{18}F]FES) Injection by Radio-Thin Layer Chromatography Analysis"
6. **GEN-Q212**, "Gas Chromatography Analysis of Residual Solvents for [^{18}F]-fluoromisonidazole [^{18}F]FMISO and [^{18}F]-fluoroestradiol [^{18}F]FES"
7. **GEN-Q113**, "Radionuclidic Identification by Half-Life Determination"
8. **GEN-Q114**, "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test"
9. **GEN-Q114A,** "Alternative procedure for Bacterial Endotoxins by Limulus Amoebocyte Lysate (LAL) Test using the PTS (Portable Test System)"
10. **GEN-Q115**, "pH Testing of Final Product"
11. **NCI-Q117**, "Sterility Test"
12. **GEN-Q117A**, " Alternative Procedure for the Sterility Test"
13. **GEN-Q110**, "Filter Integrity Test by Bubble Point"
14. **GEN-Q118**, "A Color Spot Test for the Detection of Kryptofix® [2.2.2]"
15. **GEN-Q118A**, "An Alternate Test for the Detection of Kryptofix® [2.2.2]"
16. **GEN-Q319**, "Chemical and Radiochemical Purity by HPLC for [^{18}F]FES"
17. **GEN-Q012**, "Quality Systems Administration"
18. **GEN-Q015**, "Investigating Out of Specification QC Test Results"

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19. **GEN-R004**, "Assigning Batch & Compounding Record Numbers to Production Runs"
20. **GEN-R005**, "Drug Product Complaints"
21. **GEN-R006**, "Product Labeling"
22. **GEN-R008**, "Good Documentation Practices"
23. **GEN-R209**, "QC Testing, Review, & Final Release for [^{18}F]FES"
24. **GEN-R502**, "General Equipment Maintenance"
25. **GEN-R503**, "Software Administration and Change Control for the TRACERLab FX_{F-N}"
26. **Form FQC-005**, "[^{18}F]FES Final Product QC Test Results" Form
27. **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
28. **Form MVR-001**, "Manufacturing Variance Report Form"
29. **Form EQ-001**, "General Equipment History Log"
30. **Form R004-L**, "General Log for Assigning Unique Batch or Compounding Record Numbers"
31. **Form S-FES**, "Supply Sheet for Production of [^{18}F]-Fluoroestradiol"
32. **Form DCI-L**, "Drug Product Complaint Investigation Form"
33. **Form DPC-L**, "Drug Product Complaint Log"
34. **W50ETH-L**, "Master Compounding Record for the Preparation of 50% Ethanol in Water, USP"
35. **ETH70-L**, "Master Compounding Record for Preparation of 70% Ethanol"
36. **KCO2-L**, "Master Compounding Record for the Preparation of 0.1M Potassium Carbonate"
37. **KRYP-L**, "Master Compounding Record for the Preparation of Kryptofix [2.2.2] Solution"
38. **HCL-L**, "Master compounding Record for the Preparation of 0.5 M HCl for Production of [^{18}F]FMISO and [^{18}F]FES"

B. EQUIPMENT

Equipment Description
TRACERLab FX_{F-N} System with glassware including glass reaction vial and stir bar
Cyclotron with F-18 target
HPLC Pump with Injector
Flow through UV Absorbance Detector
Vacuum Pump
Computer with TRACERlab FX_{F-N} Software installed
Printer
HPLC Preparative Column (Zorbax Phenyl 10 x 250 mm) (SS-ZOR)
Dose Calibrator (calibrated ion chamber)

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.
Graduated cylinder to measure HPLC flow, with stopwatch
Container for Solvent Waste, 500-2,000 mL bottle
Cryogen: dry ice/solvent mixture, $Nitrogen_{liq}$, or $Argon_{liq}$
Helium, Argon, or Nitrogen Gas 30-150 psig supply on and > 500 psi in tank or sufficient cryogen
Compressed Air 70-150 psig supply on with sufficient tank or compressor capacity

Reagents / Solutions	Quantity
FES supplies kit	1
FES Precursor: 3-O-Methoxymethyl-16,17-O-sulfuryl-16-epiestriol	2 ± 1 mg
Acetonitrile, USP	2.0 ± 0.2 mL
Acetone, spectrophotometric grade	qs to clean unit
$H_2{}^{18}O$, Target material	1.0 – 5.0 mL
Ethanol, absolute, USP	> 200 mL
Sterile Water for Irrigation, USP	> 300 mL
Compounded Solutions (Compounded from USP reagents)	**Quantity**
70% Vol. Ethanol	> 500 mL
(50:50) Water for injection: Ethanol (absolute) (v:v) **(ETH50-L)**	> 500 mL

D. SPECIAL NOTES AND POLICIES

- Refer to the TRACERLab FX $_{F\text{-}N}$ Operator's Guide, Chapter 2 for Health and Safety Procedures prior to operating this equipment.
- Always use non-lubricated syringes for any reagent that enters the reaction vessel.
- For loading the TRACERLab FX $_{F\text{-}N}$, use fresh, sterile syringes and needles for each solution.
- To extend the lifetime of the reagent vials you should use **only long, thin needles that reach down to the bottom of the reagent vial for the addition of small amounts of reagent.** This prevents air bubbles in the reagent vial neck; the recommendation is 23G x 2 inch or 0.60 x 60 mm. Additions can be made by piercing the septa or by unscrewing the caps and then recapping the reagent addition vials.

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-[^{18}F]FES-L**.

I. Maintenance Cleaning and Preparation of the TRACERLab FX $_{F\text{-}N}$

I.1. Condition the Preparative HPLC Column as Follows:

I.1.1. If off, turn on the compressed air and nitrogen, argon, or helium gas supplies. Verify that the gas supplies are sufficient and within the specified pressure ranges.

I.1.2. Turn on the computer to the TRACERLab and the power to the synthesizer's electronics unit. Initialize the TRACERLab software program by double-clicking on the TRACERLab icon pictured on the computer desktop.

I.1.3. The program will prompt the user to enter a password and username. All operations performed on this system require the use of a password and username.

I.1.4. Using aseptic technique, add ≥ 300 mL of 70% ethanol (**ETH70-L**), USP to eluent bottle number 2 and ≥ 500 mL of fresh preparative mobile phase (**ETH50-L)**, 50% ethanol abs, USP: 50% water for injection, USP (v:v) to eluent bottle number 1.

I.1.5. Make certain that the Zorbax phenyl semi-preparative HPLC column, **(SS-ZOR**), is correctly installed.

I.1.6. On the TRACERLab software page, select "Synthesis" on the menu bar and select "Manual" operation. Switch the injector to the inject position.

I.1.7. In manual operation, open V31 to start the mobile phase purge gas. Check that the gas is flowing at the flasks. Turn V30 to eluent 2, (70% Ethanol), Set the pump flow to 5 mL/min and run 70% ethanol (**ETH70-L**) through the column for at least 10 minutes to rinse the HPLC column and injector. Do not let the HPLC column go dry and check for leaks. If there are any leaks tighten the fittings and flow an additional 10 minutes with no leaks.

I.1.8. After the 70% ethanol has flowed through the column, set the HPLC pump flow to 0 mL/min. Turn V30 to eluent 1, (**50ETH-L**, 50% water for injection: 50% ethanol).

I.1.9. Set the pump flow to 4 mL/min. Let the HPLC pump run for at least 12 minutes. After at least 10 minutes, measure the flow rate out of the HPLC column to assure that the system is operating correctly. The measured flow rate must be between 3.6 and 4.4 mL/min. If the flow rate is incorrect purge the pump, check the fittings, and recheck flow. Once the correct flow is established, set the pump flow to 0 mL/min and close the HPLC gas purge, V31. The HPLC column will be rinsed further during the automated synthesis.

I.1.10. On the "Synthesis" tool bar, select "Stop" and then "Reset" to end manual operation.

I.2. Perform Maintenance on the TRACERLab FX $_{F\text{-}N}$ Synthesis Box as follows:

I.2.1. If necessary, remove ^{18}F separation column and purification column from synthesizer, and store the used columns in a shielded waste container to decay as per the institutional radiation waste policy.

I.2.2. Empty the contents of the ^{18}O water vial into a clean storage vial. Keep that storage vial in a shielded container to decay. Dry the ^{18}O water vial with a clean low particulate wipe and reattach it to the synthesizer.

I.2.3. Remove the cold trap from the dry ice/solvent mixture, liquid nitrogen or argon dewar. Empty any liquid from the cold trap into an approved solvent waste container. Allow the cold trap to air dry before placing it in the drying oven, if oven drying the cold trap.

I.2.4 Using appropriate safety gear fill the dewar halfway with cryogen (dry ice/solvent, liquid nitrogen or liquid argon). Replace the dewar. If using a clean cold trap from the drying oven; allow it to cool before use. Reattach the cold trap lines and carefully lower it into the dewar.

I.2.5. Empty the v-vial into an appropriate container. Dry the vial with a low particulate wipe and reattach it to the synthesizer.

I.2.6. Empty the waste bottle into an approved solvent waste container and reattach the 500 – 2,000 mL plastic waste bottle to the 3 waste lines from the synthesizer.

The waste lines must be near the top of the bottle to prevent back-flow into the synthesizer.

I.2.7. Inspect or replace the Reaction Vessel:

I.2.7.1. Pull up on the reactor until the glass reaction vessel clears the heater. Wipe the reaction vessel with a wipe to remove any excess silicone oil.

I.2.7.2. Unscrew the mounting nut and remove the reaction vessel from the reactor head. Place the reactor head on its stand.

I.2.7.3. Inspect the reaction vessel for residual liquid. The reaction vessel should be completely empty at the end of a synthesis. If there is any residual liquid from the previous synthesis, adjust the position of the reactor delivery needle and perform a reagent transfer calibration prior to using the system (Refer to the GE Service Manual).

I.2.7.4. Remove the stirring magnet from the reaction vessel.

I.2.7.5. Clean the inside of the reaction vessel and the magnet with water and ethanol so there is no visible residue. A low particulate wipe can be used. Visually inspect the reaction vessel for cracks or scratches. Visually inspect the Teflon® coating of the stir bar and replace if necessary. Dry the vessel.

I.2.7.7. Inspect the reactor head seal for cracks or scratches or liquid. If wet, dry the reactor head with a low particulate wipe. Replace the seal if necessary.

I.2.7.8. Reinstall the stir bar and the reaction vessel, and tighten the mounting nut. If needed, add 1 to 3 drops of silicone heater oil to the outside of the vessel and slide the vessel down into the heater. If oil appears at the top of the heater, pull up the reactor and remove the excess oil with a low particulate wipe.

I.2.8. Record any change in the reaction vessel or the magnetic stir bar in the Equipment History Log Book for the TRACERLab FX $_{F\text{-}N}$ Module, on **Form EQ-001**.

I.3. Cleaning the TRACERLab FX $_{F\text{-}N}$ System

I.3.1. Remove the 4 vent filters from the TRACERLab unit. Place the luer from V14 into the ~16mm X 150mm test tube to collect the acetone waste.

I.3.2. Add 1- 5 mL of acetone (**SS-ACT**), as specified, to each TRACERLab reagent Vials 1 through 6.

I.3.3. Select the **"Wash Reactor Side Only"** Program from the program menu bar. From the Synthesis menu select **"Reset."** Start the program using the **"Clean"** icon (brush) on the program's tool bar or from the **"Start"** option from the Synthesis menu or from the **"Clean"** option on the Maintenance menu. A Preparation screen will appear. Press the **ENTER** key to OK the preparation

screen. Press the **"Start Synthesis"** button. A prompt will come up asking, **"Okay to start synthesis?"** Answer **"Yes."** This will start the clean procedure. The program will cycle acetone through all the vials and the reaction vial. The cleaning procedure takes approximately 15 minutes to complete.

I.3.4. A record of the **CLEAN** program performance is automatically printed. Verify that the date and time are correct and ensure that there are no errors or functional problems encountered with the equipment prior to proceeding with the cleaning. Sign and date the printout and attach it to the batch record corresponding to this batch of FES.

II. Pre-Synthesis Setup for Manufacturing [^{18}F]FES Using TRACERLab FX_{F-N}

II.1. Prepare the ^{18}F Separation Column as follows:

II.1.1. Slowly rinse the ^{18}F Separation column (**SS-FSC**) with 1 mL of ethanol, absolute, USP, (**SS-ETH**) using a 1 mL, non-lubricated syringe.

II.1.2. Slowly rinse the ^{18}F Separation column with 1 mL of sterile water for irrigation (**SS-WIR**) using a 1 mL, non-lubricated syringe. Wait 5 minutes.

II.1.3. Expel the residual water from the column using >1 mL of air from the syringe.

II.2. Prepare the Aluminum Oxide Cartridge as Follows:

11.2.1. Wash the aluminum oxide lite cartridge with 20 mL of sterile water for irrigation by pushing water through the cartridge with a 20mL syringe and166 needle.

11.2.2. Blow the water out of the cartridge using air from the syringe and insert the cartridge between the two luer fittings near V14

11.3. Prepare the Sterile Product Vial Assembly as Follows:

II.3.1. Label the empty product sterile vial (**SS-SPV**) with the [^{18}F]FES Master Batch Number (**MBR-[^{18}F]FES-L**). Swab the top and sides of the vial. Place the vial in a clean ISO Class 5 laminar flow hood. Reswab the top of the vial using an alcohol swab.

II.3.2. Insert a venting filter needle (**SS-VFN**) into the empty sterile vial (**SS-SPV**).

II.3.3. Add 15 mL of saline for injection (**SS-NSI**), 10 mL of water for injection (**SS-WFI**) and 0.75 mL of concentrated sodium phosphates for dilution, USP (**SS-NAP**) to the vial.

II.2.4. Use aseptic technique, in a laminar flow hood, to insert a sterilizing filter (**SS-SPF**) with a ≤ 22 G needle into the final product empty sterile vial.

II.4. Attach the [^{18}F]-fluoride line from the cyclotron target to the [^{18}F] inlet line of the synthesis box or confirm that this line is attached.

II.5. Add 0.1 M K_2CO_3 Solution as follows:

II.5.1. Draw up 0.50 ± 0.05 mL of 0.1 M K_2CO_3 (**KCO2-L**) solution using a 1.0 mL, non-lubricated, syringe and long needle and transfer it to vial 1 of the synthesis box. The needles can be pushed through the reagent vial septa or the reagent vials can be uncapped (preferred) for reagent addition. Make certain the caps on the glass vials are tight after the additions.

II.6. Add Kryptofix [2.2.2] solution as follows:

II.6.1. Draw up 2.0 ± 0.2 mL of 15 mg/ 2 mL Kryptofix [2.2.2] (**KRYP-L**) in anhydrous acetonitrile using a 3.0 mL, non-lubricated syringe with long needle. Transfer the solution to vial 2 of the synthesis box.

II.7. Add 0.5 M HCl Solution as follows:

II.7.1. Draw up 1.0 ± 0.05 mL of 0.5 M HCl (**HCL-L**) using a 1.0 mL, non-lubricated syringe and long needle. Transfer the solution to vial 4 of the synthesis box.

II.8. Add Sodium Bicarbonate / Ethanol Solution as follows:

II.8.1. Using aseptic technique, draw up 3.0 ± 0.3 mL of the 50% mobile phase (**ETH50-L**) using a 3.0 mL, non-lubricated, syringe and short needle. Transfer the solution to vial 5 of the synthesis box.

II.8.2. Then, using aseptic technique, draw up 1.2 ± 0.1 mL of USP sodium bicarbonate solution (**SS-BIC**) using a 3.0 mL, non-lubricated, syringe and short needle. Transfer the solution to vial 5 of the synthesis box.

II.9. Prepare and add the FES precursor Solution as follows:

II.9.1. Draw up 1.5 ± 0.1 mL of anhydrous acetonitrile (**SS-ACN**) using a 3.0 mL, non-lubricated syringe and long needle.

II.9.2. Add the acetonitrile to the vial containing 2 mg of the FES precursor, 3-O-methoxymethyl-16,17-O-sulfuryl-16-epiestriol (**SS-MSE**), through the septum and shake the vial to dissolve.

II.9.3. Leave the syringe in the vial and the vial as it is until it is time to add to the vial. Transfer the dissolved contents into the TRACERLab reagent Vial 3 using the 3 mL non-lubricated syringe and long needle.

II.10. Run the FES Program as follows:

II.10.1. The Method for the synthesizer to use must be selected first. From the icon line, verify the "NCIFES15V1" method appears in the methods window. Select the synthesis menu and reset the program using a hard button reset and then the software reset. Select the blue "**I**" synthesis Icon or in the synthesis menu and then press "Start" to start the synthesis.

II.10.2. A window entitled "Preparation" will open showing the steps to be followed After each instruction is completed or verified to be complete, press the **ENTER** key. The steps for the preparation screen are:

II.10.2.1. Prompt: "Enter the batch number." The operator should enter the unique identifying batch number for the synthesis, per **GEN-R004**, and then presses the **ENTER** key to confirm.

II.10.2.2. Prompt "Connect the gas bags (optional)." Connect any radioactive gas traps to the unit, if they are used.

II.10.2.3. Prompt: "Check that the cold trap fittings are tight and that there is cryogen in the dewar." This was completed in steps I.2.4, verify this by pressing the **ENTER** key.

II.10.2.4. Prompt: "Make certain that the inert gas and air supplies are on." This was completed in steps I.1.1, verify this by pressing the **ENTER** key.

II.10.2.5. Prompt: "Attach 4 sterile vent filters (**SS-SVF**) to box if they are off." The operator should verify that the 4 vent filters are installed and press the **ENTER** key.

II.10.2.6. Prompt: "Confirm that the ^{18}F line from the target is attached to the box." Verify that this step was done as required in section II.4 and press the **ENTER** key.

II.10.2.7. Prompt: "Attach F-18 separation cartridge at luer 1 position." The operator should attach the F-18 separation cartridge (**SS-FSC**), which was prepared as part of step II.1, to the luer 1 position of the box. The operator then presses the **ENTER** key to confirm that this step is complete.

II.10.2.8. Prompt: "Add 0.5 mL of 0.1 M K_2CO_3 (**KCO2-L**) to Vial 1." The operator should verify that step II.5 was completed by pressing the **ENTER** key.

II.10.2.9. Prompt: "Add 2.0 mL of 15 mg / 2.0 mL Kryptofix® [2.2.2] (**KRYP-L**) in CH_3CN to Vial 2." The operator should verify that step II.6 was completed by pressing the **ENTER** key.

II.10.2.10. Prompt: "Add 1.0 mL of 0.5 M HCl solution (**HCL-L**) to Vial 4." The operator should verify that step II.7 was completed by pressing the **ENTER** key.

II.10.2.11. Prompt: "Add 3.0 mL of 50% ethanol and 1.2 mL of sodium bicarbonate solution to vial 5." The operator should verify that the addition of sodium bicarbonate from step II.8 was completed by pressing the **ENTER** key.

II.10.2.12. Prompt: "Connect the luer from V14 connected through the Al_2O_3 Cartridge." The operator should make certain that the

Al_2O_3 cartridge is installed correctly between the luer fittings from V14 as per step II.2. The operator should also make certain that the tubing connection from the round-bottom flask to the Column 3 connector is connected without any column. The operator then should press the **ENTER** key to confirm that this step is complete.

II.10.2.13. Prompt: "Connect the luer from HPLC product line to the product collection vial." The operator should connect the product collection vial from from step II.3 through the sterilizing filter to the male luer fitting from the HPLC product collection line (V18). The operator then should verify that the step is completed by pressing the **ENTER** key.

II.10.2.14. Prompt: "Verify > 300 mL of mobile phase is in the eluent 1 bottle." The operator should verify that at least 300 mL of the **ETH50-L** mobile phase remains in the eluent 1 bottle and then press the **ENTER** key.

II.10.2.15. Prompt: "Add 2 mg FES precursor (**SS-MSE**) in 1.5 mL anhydrous CH3CN (**SS-ACN**) to Vial 3." The operator should verify that step II.9 was completed by pressing the **ENTER** key.

II.10.2.16. Prompt: "Enter operator initials." The operator should enter their initials.

III. Manufacturing [^{18}F]FES Using TRACERLab FX $_{F\text{-}N}$

III.1. Synthesize [^{18}F]FES

III.1.1. After the requested inputs are made, press the "**START SYNTHESIS**" button to start the synthesis. Answer the prompt "Okay to start synthesis?" by pressing "yes."

III.1.2. From the cyclotron control station, deliver the $^{18}F^-$ activity to the synthesis box. Observe the accumulation of $^{18}F^-$ activity in the v-vial. Allow activity to transfer from the target until the v-vial activity ceases to increase. Record the times that $^{18}F^-$ collection started and stopped.

III.1.3. Verify that the cyclotron transfer process is complete on the cyclotron workstation. Then on the synthesizer computer, press the "**Click okay after F$^-$ is delivered from cyclotron**" button a second time. The synthesis begins with the time list window and the reaction parameter chart graphs displayed. The synthesis program will take approximately 30 minutes to complete. Record synthesis start and stop times, and the amount of ^{18}F delivered in the corresponding master batch record. It is allowable to pause the program if additions are going too slow, but a variance report should be noted and the frits on the addition vials checked after the synthesis.

III.1.4. Once the synthesis is complete, the product is injected on the HPLC column (**SS-ZOR**) for purification. The software display will change to the HPLC detector output screens once the product has been injected onto the HPLC system. Both the UV and Radioactivity traces are displayed. The eluting [^{18}F]FES should be visible on the Radioactivity display at approximately 9 minutes (flow rate is 4.0 mL/min). Press the "**COLLECT**" Icon when the FES peak appears on the Radioisotope display. When the peak is no longer visible press the "**STOP COLLECT**" Icon to end the collection (> 1 minute later). Record the start and stop chromatographic retention times.

III.1.5. With the Process control screen displayed, the end of the FES program (~ 5 minutes after product collection) is signified by hyphens in the status box. Remove the sterilizing filter from the [^{18}F]FES product vial.

III.1.6. Remove the shielded [^{18}F]FES product vial assembly from the hot cell if necessary.

III.1.7. A performance record of the FES program will automatically be printed. Verify that the assigned batch number is correct. Sign and date the printout and attach it to the corresponding batch record for this batch of final product.

IV. Post Synthesis

IV.1. Withdraw Samples for Quality Control Testing

IV.1.1. 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.

IV.1.2. Visually inspect the product [^{18}F]FES as per **GEN-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-005**.

IV.1.3. Detach the sterilizing filter from the delivery line, and place it in a lead shield for the filter integrity test. Rinse the filter with 10 mL of water for irrigation to increase the surface tension on the membrane (50% ethanol surface tension is too low). Perform the integrity test as per SOP **GEN-Q110** and record the bubble point pressure on the batch record.

IV.1.4. Swirl the vial to completely mix the contents. Swab the product [^{18}F]FES vial with an alcohol swab. Using a 0.5 mL tuberculin syringe, aseptically withdraw an accurate ~0.5 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-005**.

IV.1.5. In the batch record, calculate the specific concentration in mCi/mL, at the End of Synthesis (EOS).

IV.1.6. Aseptically use a (~ 22G) x 1½" needle on a 1 (or 3) mL syringe to withdraw a >1.0 mL sample into the syringe for sterility tests (1.0 mL straight or 0.5mL can be used with an additional 0.5 mL of water for injection) and reserve sample (>0.5mL) 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.

IV.1.7. Record the results of the QC release tests on **Form FQC-005**. The tests to be conducted should include the following:

- "Radiochemical Identification & Purity of [^{18}F]-Fluoroestradiol ([^{18}F]FES) Injection by Radio-Thin Layer Chromatography Analysis," per SOP **GEN-Q311**
- "Gas Chromatography Analysis of Residual Solvents for [^{18}F]-fluoroestradiol [^{18}F]FES and [^{18}F]-fluoroestradiol [^{18}F]FES," per SOP **GEN-Q212**
- "Radionuclidic Identification by Half-Life Determination," per SOP **GEN-Q113**
- "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test," per SOP **GEN-Q114 or** "Alternative Procedure for Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test Using the PTS (Portable Test System), " per SOP **GEN-Q114A.**
- "pH Testing of Final Product," per SOP **GEN-Q115**
- "A Color Spot Test for the Detection of Kryptofix® [2.2.2]," per SOP **GEN-Q118** or "An Alternative Test for the Detection of Kryptofix® [2.2.2] per SOP **GEN-Q118A**.
- "Chemical and Radiochemical Purity by HPLC for [^{18}F]FES," per SOP **GEN-Q319**
- "Chemical Purity by Visual Inspection," per SOP **GEN-Q121**

IV.1.8. Record any manufacturing variances as per SOP **GEN-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.

IV.1.9. Attach appropriate labels to the [^{18}F]FES product vial and in the batch production record where indicated.

IV.1.10. Perform final review and release of QC test results as per **GEN-R209**, "QC Testing, Review, & Final Release for [^{18}F]FES."

IV.1.11. Record final sterility results on **Form FQC-005** once they are complete and available. The sterility testing should be completed as per "Sterility Test," per SOP **GEN-Q117 or** "Alternative Procedure for the Sterility Test," as per SOP **GEN-Q117A.**

V. Clean-Up of the TRACERLab FX $_{F-N}$ System Post-FES Synthesis

V.1. Clean the HPLC Injector & Column with Ethanol

V.1.1. Make certain that the lines from V18, V15, and VICI2 (injector) are attached to the waste bottle.

V.1.2. Add 70% ethanol (**ETH70-L**, 4 mL) to TRACERLab HPLC loading vial by passing the alcohol through the female luer side of V14. Reconnect the V14 luer fitting. Shake the vial for >15 sec. Place the end of the HPLC product line fitting into the glass waste tube.

V.1.3. Change out the eluent bottle 1 containing the preparative mobile phase (**ETH50-L**), 50% water for injection and 50% absolute ethanol to a new eluent bottle 1 containing >300 mL sterile water for irrigation (**SS-WIR**).

V.1.4. On the TRACERLab software page, select "Synthesis" on the menu bar and select "Manual" operation. Switch the injector to the inject position.

V.1.5. In manual operation, open V31 to start the mobile phase purge gas. Check that the gas is flowing at the flasks. Turn V30 to eluent 1, water for irrigation (**SS-WIR**).

V.1.6. Set the pump flow to 5 mL/min and run water for irrigation (**SS-WIR**) through the column for at least 10 minutes to rinse the HPLC column and injector of salts.

V.1.7. After the water has flowed through the column, set the HPLC pump flow to 0 mL/min.

V.1.8. Replace the eluent 1 bottle with > 200 mL of absolute ethanol (**SS-ETH**).

V.1.9. Set the pump flow to 5 mL/min and run absolute ethanol (**SS-ETH**) through the column for at least 10 minutes to rinse the HPLC column and injector of very lipophilic compounds.

V.1.10. Set the HPLC pump flow to 0 mL/min and close the HPLC gas purge, V31. The HPLC column will be rinsed further during the automated synthesis. On the "Synthesis" tool bar, select "Stop" to end manual operation.

V.1.11. Change out the eluent bottle 1 to a new eluent bottle 1 containing >200 mL of 70% ethanol (**ETH70-L**).

V.1.12. Select the TRACERLab FES HPLC cleaning Program, "FES HPLC Clean." Reset the program under the synthesis menu.

V.1.13. Start the FES HPLC cleaning program.

V.1.14. Verify the cleaning program is complete.

V.1.15. Attach a new sterilizing filter (**SS-SPF**) to the luer fitting on V18 (b side) on the product HPLC outlet.

V.1.16. Sign and date print out, and attach it to the batch record. Document any work on the equipment in the equipment history log book on **Form EQ-001**.

V.2. Clean the TRACERLab FX $_{F\text{-}N}$ Box

V.2.1. Remove the aluminum oxide cartridge. Insert the V14 luer line (male side) into the glass waste test tube. Add ~ 5 mL of water for irrigation to Vial 5 and ~3 mL of water for irrigation to Vial 4 using a 10 cc non-lubricated syringe and short needle. Then select manual operation and sequentially open V14, open V5, open V19 and switch V12 to the right position. When all of the water has transferred out of the vial to the reaction vessel, close V5, lower the reaction vial "needle" (PEEK transfer tube) and open V4. When all of the liquid is in the glass waste tube, the operator should close V19, close V4, return the "needle" to the up position, switch V12 to left and close V14. On the menu press the "Stop" to end manual operation.

V.2.2. On the "Synthesis" menu bar, select the "Wash Reactor Side Only program", reset the program.

V.2.3. Remove the 4 vent filters (Vial 1, Vial 2, V21 Exhaust, V26 Exhaust) from the TRACERLab unit. Remove the fluoride (F-18) separation cartridge from luer one position.

V.2.4. Add 1 to 5 mL of acetone (**SS-ACT**), as specified, to each TRACERLab reagent Vials 1 through 6.

V.2.5. Start the cleaning program using the **"Clean"** icon (brush) on the program's tool bar or from the **"Start"** option from the Synthesis menu or from the **"Clean"** option on the Maintenance menu. A Preparation screen will appear. Enter the batch number for cleaning and answer all prompts on preparation list by pressing the **ENTER** key. Press the **"Start Synthesis"** button. A prompt will come up

asking, **"Okay to start synthesis?"** Answer **"Yes."** This will start the cleaning procedure.

V.2.6. Verify the cleaning program is completed. Verify that the date and time on the report are correct and ensure that there are no errors or functional problems encountered with the equipment prior to proceeding with the cleaning. Sign and date the printout and attach it to the batch record corresponding to this batch of FES.

V.2.7. Attach 4 new vent filters (**SS-SVF**) to the TRACERLab box. Reconnect the V14 luer.

V.2.8. Empty the waste container.

V.2.9. Close down the TRACERLab program.

V.2.10**.** Shut down the computer and turn off the power to the module. Empty the cold trap and the dewar.