Template Provided By:

Cancer Imaging Program

National Cancer Institute

[^{18}F] Fluoroestradiol: [^{18}F]FES Injection Master Batch Record

[^{18}F]FES Batch No.: MBR-FES-_____ Manufacturing Date: _____(Expires 12 hr EOS)

Equipment Description	Internal Tracking Number/ Identification	Manufacturer	Verified By (Initials)
TRACERLab FX $_{F-N}$ System with glassware including glass reaction vial and stir bar		GE Medical Systems	
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)			

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.	
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	
Others from GEN-MPR	

Reagents / Compounded Solutions	Internal Tracking Number/ Identification	Quantity	Verified By (Initials)
FES supplies kit	**Form-S-FES-_____**	1	
FES Precursor, 3-O-Methoxymethyl-16,17-O-sulfuryl-16-epiestriol	**SS-MSE-_____**	2 ± 1 mg	
Acetonitrile, anhydrous	**SS-ACN-_____**	2.0 ± 0.2 mL	
Acetone, spectrophotometric grade	**SS-ACT-_____**	qs to clean unit	
$H_2{}^{18}O$, Target material	**SS-O18-_____**	1.0 to 5.0 mL	
Ethanol, absolute (USP)	**SS-ETH_____**	> 200 mL	
Water for Irrigation (to rinse HPLC, FSC and Al_2O_3)	**SS-WIR-_____**	> 300 mL	
70% Vol. Ethanol (HPLC disinfecting solution)	**ETH70-_____**	≥ 500 mL	
50% Vol. Ethanol (preparative mobile phase)	**ETH50-_____**	> 500 mL	

GEN-[^{18}F]Fluoroestradiol: [^{18}F]FES Injection Master Batch Record Version: **V0**

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

Regulatory Approval:_____**Date:** _____ .
Signature

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(Initial all blanks when the step is performed as listed, add values or "no" where appropriate.)

I. Maintenance and Cleaning of the TRACERLab FX $_{F-N}$	**Performed By Initial/Date**
I.1 Conditioning the Preparative HPLC Column (Requires ~30 min)	
I.1.1. N_2, He or Ar supply pressure _____ psi (30–150). Air pressure_____ psi (70–150).	_____
I.1.2. TRACERLab module power on. Computer on and Windows software initialized.	_____
I.1.3. TRACERLab software initialized, enter user name and password.	_____
I.1.4 Add ≥ 300 mL of 70% ethanol, (**ETH70-L**), to eluent bottle 2 and ≥ 500 mL of preparative mobile phase (50% ethanol in water for injection, (**ETH50-L**) to eluent bottle 1 70% Ethanol: volume added: _____mL (≥ 300 mL) 50% Ethanol Mobile Phase: volume added: _____ mL (≥ 500 mL)	_____
I.1.5. Make certain that the Zorbax preparative HPLC column (**SS-ZOR**) is installed.	_____
I.1.6 Select manual operation from the software menu and switch the injector to inject position.	_____
I.1.7. Open the He or N_2 eluent purge. Turn valve 30 to eluent 2 (70% ethanol). Set pump flow to 5 (mL/min), _____, Let it run for > 10 minutes, _____ (minutes run).	_____
I.1.8. Set pump flow to 0 (mL/min), _____, Turn valve 30 back to eluent 1 (50% ethanol)	_____
I.1.9. Set pump flow to 4 (mL/min), _____, Let it run for > 12 minutes, _____ (minutes run). Check that the flow is steady. Measured flow after ~10 minutes is: _____ (mL/min)(must be between 3.7 and 4.3). Set the pump flow to 0 (mL/min), _____ and close the He, Ar or N_2 eluent purge (V30).	_____
I.1.10. Go to the software menu bar and select manual stop. Reset the program.	_____
I.2. Perform Maintenance on the TRACERLab FX $_{F-N}$ Synthesis Box.	
I.2.1. Remove any used ^{18}F separation and alumina purification columns from the synthesizer.	_____
I.2.2. Empty the ^{18}O water vial, dry it and reattach it to the synthesizer.	_____
I.2.3. Remove, empty and replace the cold trap.	_____
I.2.4. Fill the dewar with new cryogen. Reattach clean cold trap.	_____
I.2.5. Empty then dry the v-vial and reattach it to the synthesizer.	_____
I.2.6. Empty the waste bottle. Verify that the lines from V18, V15, and VICI2 (Injector) are attached to the waste bottle.	_____
I.2.7. Inspect or replace the Reaction Vessel, clean with water and ethanol, USP, dry the vessel. • Residual liquid in Reaction Vessel from previous synthesis _____ mL. Delivery tube: position _____ position adjusted _____ tube replaced _____ • Magnetic stirring bar condition _____, bar replaced _____. • Reaction vessel condition _____, vessel replaced _____. Heater oil added _____.	_____
I.2.8. If the reaction vessel or magnetic stir bar are replaced, record the equipment maintenance in the equipment history log book, on **Form EQ-001**.	_____
I.3. Cleaning the TRACERLab FX $_{F-N}$ System	
I.3.1. Remove the 4 vent filters from the TRACERLab unit. Remove and place the luer from V14 into a test tube (~16 X 150 mm) to collect the acetone waste.	_____
I.3.2. Add acetone (**SS-ACT**) to TRACERLab reagent Vials (volumes are approximate, use a 10 mL nonlubricated syringe with short needle, save syringe and needle to use in step V): Vial 1 _____ mL (1 mL), Vial 2 _____ mL (2 mL), Vial 3 _____ mL (2 mL), Vial 4 _____ mL (2 mL), Vial 5 _____ mL (5 mL), Vial 6 _____ mL (1 mL).	_____
I.3.3. Start the "Wash Reactor Side Only" Program. o Go to the synthesis menu and select reset; then start the cleaning program. o Start the synthesis when prompt shows "Okay to start synthesis", press yes. o Verify the cleaning program is completed.	_____
I.3.4. Sign and date the print out, and attach it to this record.	_____

II. Pre-Synthesis Setup for Manufacturing [^{18}F]FES Using TRACERLab FX(takes ~20 min)	Performed By Initial/Date
II.1. Prepare the ^{18}F Separation Cartridge (**SS-FSC**), use two 1 mL non lubricated syringes with short needles Slowly wash cartridge with 1 mL of ethanol (**SS-ETH**). Slowly wash cartridge with 1 mL of sterile water for irrigation (**SS-WIR**) do not blow out, wait 5 minutes. Push air through the column to expel the residual water.	 _____ _____ _____
II.2. Prepare the Aluminum Oxide Cartridge: (20 ml syringe and 16 G needle) Wash the aluminum oxide lite cartridge with 20 ml of sterile water for irrigation. Blow out the cartridge and insert it between the two luer fittings near V14.	 _____ _____
II.3. Prepare the Sterile product vial assembly: (regular lubricated syringes and needles) Use aseptic technique in the laminar air flow hood to do the following tasks: Label the empty product sterile vial (**SS-SBV**) with "[^{18}F]-FES" batch number. Swab the vial. Insert a venting filter needle (**SS-VFN**) into the empty sterile vial (**SS-SBV**). Add 15 mL of saline for inj, (**SS-NSI**), 10 mL of water for inj (**SS-WFI**), and 0.75 mL of conc PBS for dilution (**SS-NAP**), all USP to the vial using a 20 cc syringe, a 10 cc syringe and a 1.0 cc syringe. Reswab vial. Insert a sterilizing filter (**SS-SPF**) with a 22G needle into the final product empty sterile vial.	 _____ _____ _____ _____ _____
II.4. Make certain the ^{18}F target line is hooked up to the TRACERLab FES automation unit	_____
II.5. 0.1 M K_2CO_3 Solution Addition: (Use 1 ml nonlubricated syringe and long needle) Transfer 0.50 ± 0.05 mL 0.1 M K_2CO_3 (**KCO2-L**) to box vial 1.	_____
II.6. Kryptofix® [2.2.2] addition: (Use 3 ml nonlubricated syringe and long needle) Transfer 2.0 ± 0.2 mL of Kryptofix® [2.2.2] (**KRYP-L**) 15 mg/ 2 mL in acetonitrile to box vial 2.	_____
II.7. HCl addition: (Use 1 ml nonlubricated syringe and long needle) Transfer 1.0 mL ± 0.05 mL of 0.5 M HCl (**HCL-L**) to box vial 4	_____
II.8. Sodium bicarbonate / ethanol addition: (Use 3 ml nonlubricated syringes and short needles) Aseptically transfer 3.0 ± 0.3 mL of 50% ethanol mobile phase (**ETH50-L**) to vial 5. Then aseptically add 1.2 ± 0.1 mL of sodium bicarbonate (**SS-BIC**) to the same vial 5.	_____
II.9. FES precursor addition: (Use 3 cc nonlubricated syringe and long needle) Aseptically, transfer 2.0 ± 0.2 mg FES precursor (**SS-MSE**) in 1.5 ± 0.1 mL CH_3CN to vial 3.	_____
II.9. Run the FES Program: Select the Synthesizer METHOD, "NCIFES15V1" from the icon line. Under synthesis menu select RESET after doing a hardware reset. Then under the synthesis menu press "Start" to start the synthesis. The "Preparation" window will appear:	_____

Preparation Step	*Answer*
Enter the Batch number	*Enter batch #*
Connect gas bags (optional)	OK
Check that the cold trap fittings are tight and that there is cryogen in the dewar	OK
Make certain that the inert gas and air supplies are on	OK
Attach 4 vent filters (SS-SVF) to box if they are off	OK
Confirm that the F18 line from the target is attached to the box	OK
Attach F-18 separation cartridge (SS-FSC) at luer 1 position	OK
Add 0.5 mL of 0.1 M K2CO3 (KCO2-L) to Vial 1	OK
Add 2.0 mL of 15 mg / 2.0 mL Kryptofix [2.2.2] (KRYP-L) in CH3CN to Vial 2	OK
Add 1.0 mL of 0.5 M HCl solution (HCL-L) to Vial 4	OK
Add 3.0 mL of 50% ethanol and 1.2 mL of sodium bicarbonate solution to vial 5	OK
Connect the luer from V14 connected through the Al_2O_3 cartridge	OK
Connect the luer from HPLC product line to the product collection vial	OK
Verify > 300 mL of mobile phase is in the eluent 1 bottle (ETH50-L)	OK
Add 2 mg FES precursor (SS-MSE) in 1.5 mL anhydrous CH3CN (SS-ACN) to Vial 3	OK
Enter operator initials	Enter initials

III. Manufacturing [^{18}F]FES Using TRACERLab FX $_{F-N}$	**Performed By Initial/Date**
III.1. Synthesize [^{18}F]FES	
III.1.1. Press the "**START SYNTHESIS**" button. "Okay to start synthesis?" Press "yes."	_____
III.1.2. Deliver the $^{18}F^-$ activity to the synthesis box.	_____
$^{18}F^-$ Delivery: Start Time: _____ Time Complete: _____ (time of day)	_____
III.1.3. Verify cyclotron transfer of ^{18}O water (**SS-O18**) with $^{18}F^-$ process ^{18}F fluoride is complete. Press the "**okay"** button for the prompt**:** "Click okay after F^- is delivered from cyclotron."	_____
Synthesis: Start Time: _____ Stop Time: _____ (time of day) **Quantity of ^{18}F Delivered:** _____mCi	
Note any pauses due to slow reagent addition here:_____	
III.1.4. HPLC Collection: Press the "**COLLECT**" Icon when the FES peak starts on the Radioactivity trace.	_____
HPLC Fraction Started: _____ minutes (Retention Time)	
Press the "**STOP COLLECT**" Icon to end the [^{18}F]FES product collection.	
HPLC Fraction Stopped: _____ minutes (Retention Time)	
III.1.5. 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. Verify the assigned batch number, sign and date the printout, and attach it to this batch record	_____

IV. Post-Synthesis	**Performed By Initial/Date**
IV.1. Withdraw Samples for Quality Control Testing	_____
IV.1.1. Assay the [^{18}F]FES product using a calibrated ion chamber.	_____
Final Product Assay: _____ mCi Time of End of Synthesis (EOS): _____ (time of day)	
IV.1.2. Particulate test of the final product as per **GEN-Q121**, (describe), _____.	
IV.1.3. Perform an integrity test of the 10 mL water for irrigation wetted sterile product filter as per **GEN-Q110**. Record the results of the filter integrity test here, _____ psi.	_____ _____
IV.1.4. Mix vial by **swirling**, sample Withdrawn for QC Testing: _____ mL (0.5 mL)	_____
Assay of Quality Control Sample: _____ mCi at _____ (time of day).	
Calculated EOS Activity: **Sample mCi / $e^{-\lambda t}$ = _____ mCi (EOS)** **$e^{-\lambda t}$ = _____**	
(where λ = ln(2)/109.8 and t = (Assay Time – EOS) in minutes)	
IV.1.5. Calculated Specific Concentration: = **mCi (EOS) / mL sample = _____ mCi/mL**	
Calculated Final Product Volume: = **Final Product Assay / Calculated Specific Concentration = _____ mL (30-35 mL)**	_____
IV.1.6. Sterility and Reserve Samples Withdrawn: _____ mL (1 (or 3) mL, use short needle)	_____
IV.1.7. Record the results of the QC release tests on **Form FQC-005** as they are obtained.	_____
IV.1.8. Attach all manufacturing variances to the batch record. Record the # of variances reported here, _____.	_____ _____
IV.1.9. Attach appropriate labels to the product vial. Also attach exact copies of the labels placed on the final product dose vials (syringes) below in the batch record for documentation of the labeling that was used for doses for this batch of product.	
IV.1.10. Perform final review and release of QC Test Results as per **GEN-R309**.	_____
IV.1.11. Record final sterility results on **Form FQC-005** once they are available	_____

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

Place Copies of Labels Here
(if extra room is needed put labels on the back of this sheet)

V. Post-Synthesis Clean-Up of [^{18}F]FES System

V.1. Cleaning the HPLC Injector & Column

_____**V.1.1.** Attach the lines to the waste bottle.

_____**V.1.2.** Add 70% ethanol (**ETH70-L**, 4 mL) to Synthesis Module HPLC loading vial _____ mL through the V14 luer. Reconnect the V14 luer fitting. Use a 5 mL regular syringe with a short needle. Shake the vial for at least 15 seconds. Place the outlet of the HPLC product line in the glass waste tube.

_____**V.1.3.** Add ≥ 300 mL of sterile water for irrigation, (**SS-WIR**), to eluent bottle 1

Internal Tracking No: SS-WIR-_____ Volume Added: _____ mL (≥ 300 mL**)**

_____**V.1.4.** Select manual operation from the software menu bar and switch the injector to the inject position.

_____**V.1.5.** Open the He or N_2 eluent purge (V31). Turn the valve to eluent 1 (water for irrigation)(V30).

_____**V.1.6.** Set pump flow to 5 (mL/min), _____, Let it run for > 10 minutes, _____ (minutes run).

_____**V.1.7.** Set pump flow to 0 (mL/min), _____,

_____**V.1.8.** Add ≥ 200 mL of 100% ethanol, to eluent bottle 1

100% Ethanol Internal Tracking No: ETH-_____ Volume Added: _____ mL (≥ 200 mL**)**

_____**V.1.9.** Set pump flow to 5 (mL/min), _____, Let it run for > 10 minutes, _____ (minutes run).

_____**V.1.10**. Set pump flow to 0 (mL/min), _____, Stop the program. _____.

_____**V.1.11.** Change eluent bottle 1 to contain > 200 mL of 70 % Ethanol (**ETH70-L**), _____ mL.

_____**V.1.12.** Select FES HPLC Cleaning Program "FES HPLC Clean". Reset the program under the synthesis menu.

_____**V.1.13.** Start the HPLC FES cleaning program.

_____**V.1.14.** Verify the cleaning program is complete.

_____**V.1.15.** Attach a new sterilizing filter **(SS-SPF)** on the product HPLC outlet.

_____**V.1.16.** Sign and date printout, 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. Cleaning the TRACERLab FX System (reagent and reaction vials)

_____**V.2.1**. Remove the aluminum oxide cartridge. Put the V14 luer line 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 (use a 10 cc nonlubricated syringe and short needle). Select manual operation. Open V14, open V5, open V19, switch V12 to right. When the liquid is in the reaction vessel, close V5. Set needle down. Open V4. When all of the liquid is in the tube, close V19, close V4, return needle up, V12 left and V14 closed. Stop manual operation.

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

_____**V.2.3**. Remove the 4 filters from Vial 1, Vial 2, V21 exhaust, V26 exhaust. Remove the F-18 separation cartridge from luer 1 position.

_____**V.2.4**. Add acetone (**SS-ACT**) to TRACERLab reagent Vials:

Vial 1 ____ mL (1 mL), Vial 2 ____ mL (2 mL), Vial 3 _____ mL (2 mL),
Vial 4 _____ mL (2 mL), Vial 5 _____ mL (5 mL), Vial 6 _____ mL (1 mL).

_____**V.2.5**. Start the "Wash Reactor Side Only" Program.(using time list: "Wash reactor side only". Answer all prompts on preparation list. Start synthesis when prompt shows "Okay to start synthesis", press yes_____.

_____**V.2.6.** Verify the cleaning program has ended. Print, sign and date the cleaning report. Attach it to this record.
_____**V.2.7**. Attach 4 new vent filters (**SS-SVF**) to the synthesis unit. 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.

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

We certify that [^{18}F]-Fluoroestradiol Production Batch **MBR-[^{18}F]FES-**_____ was prepared according to the Manufacturing Instructions in the Manufacturing Production Record **MPR-[^{18}F]FES**. Any additional manufacturing modifications have been captured along with the appropriate control information as outlined in **NCI-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 **GEN-R309**, "QC Testing, Review, and Final Release for [^{18}F]FES" 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 **GEN-Q117** or **GEN-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 **GEN-R309**, "QC Testing, Review, and Final Release for [^{18}F]FES," and the OOS investigation as per **GEN-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 [^{18}F]-Fluoroestradiol Production Batch **MBR-[^{18}F]FES-**_____:

_____ 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