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Master Production Record

MPR-[^{18}F]FLT

For Synthesis of 3'-Deoxy-3'-[^{18}F]Fluorothymidine: [^{18}F]FLT Injection

Approval Signatures:

Printed Name	Date	Signature	Date
Author:			
Supervisor:			
Regulatory Affairs/QA:			

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Manufacture of [^{18}F]FLT Using a Nucleophilic Synthesis Module

Purpose: This is the Master Production Record for synthesizing 3'-Deoxy-3'-[^{18}F]Fluorothymidine using a Nucleophilic Synthesis Module, an automated radiochemistry system, to produce this specific fluorine-18 labeled radiopharmaceutical product for investigational use only.

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Manufacture of [^{18}F]FLT Using a Nucleophilic Synthesis Module

REFERENCES & MATERIALS OVERVIEW

A. REFERENCES

1. **GEN-Q121,** "Chemical Purity by Visual Inspection**"**
2. **Nucleophilic Synthesis Module Operator Guide (**to be provided by the sponsor**)**
3. **Nucleophilic Synthesis Module Software Manual**
4. **Cyclotron Operator Manual**
5. **GEN-Q111,** "Radiochemical Identification & Purity of 3'-Deoxy-3'-[^{18}F]Fluorothymidine [^{18}F]FLT Injection by Radio-Thin Layer Chromatography Analysis"
6. **GEN-Q112**, "Gas Chromatography Analysis of Residual Solvents"
7. **GEN-Q113**, "Radionuclidic Identification by Half-Life Determination"
8. **GEN-Q114**, "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test"
9. **GEN-Q115**, "pH Testing of Final Product"
10. **GEN-Q117**, "Sterility Test"
11. **GEN-Q110**, "Filter Integrity Test by Bubble Point"
12. **GEN-Q118**, "A Color Spot Test for the Detection of Kryptofix® [2.2.2] in [^{18}F]FLT"
13. **GEN-Q119**, "Chemical and Radiochemical Purity by HPLC"
14. **GEN-Q012**, "Quality Systems Administration"
15. **GEN-Q015**, "Investigating Out of Specification QC Test Results"
16. **GEN-R004**, "Assigning Batch & Compounding Record Numbers to Production Runs"
17. **GEN-R005**, "Drug Product Complaints"
18. **GEN-R006**, "Product Labeling"
19. **GEN-R008**, "Good Documentation Practices"
20. **GEN-R009**, "QC Testing, Review, & Final Release"
21. **GEN-R502**, "General Equipment Maintenance"
22. **GEN-R503**, "Software Administration and Change Control for the Nucleophilic Synthesis Module"
23. **Form FQC-001**, "[^{18}F]FLT Final Product QC Test Results" Form I
24. **Form FQC-002**, "Final Product QC Testing" Form II
25. **Form IOS-001**, "Investigating Out of Specification (OOS) QC Test Results Form"
26. **Form MVR-001**, "Manufacturing Variance Report Form"
27. **Form EQ-001**, "General Equipment History Log"
28. **Form R004-L**, "General Log for Assigning Unique Batch or Compounding Record Numbers"
29. **Form DCI-L**, "Drug Product Complaint Investigation Form"
30. **Form DPC-L**, "Drug Product Complaint Log"
31. **HBS-L**, Compounding Record for "Preparation of HPLC Buffer Solution for Purification of [^{18}F]FLT"

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32. KCO-L, Preparation of 0.04 M (K_2CO_3) Potassium Carbonate Solution
33. NAH-SOLN-L, Preparation of 1% (NaOH) Sodium Hydroxide Solution
34. ETH70-L, Preparation of 70% Ethanol

B. EQUIPMENT

Equipment Description	Equipment Identification Number	Manufacturer	Quantity
Cyclotron			
Nucleophilic Synthesis Module with glassware			
HPLC Pump with Injector			
Flow through UV Absorbance Detector			
Vacuum Pump			
Computer with Nucleophilic Synthesis Module Software installed			
Printer			
Shielding for Nucleophilic Synthesis Module / Hot Cell			

C. REAGENTS/MATERIALS

GENERAL (Non-Inventoried per Batch) SUPPLIES

Supply Description
Appropriate safety wear including radiation badges, lab coat, safety glasses, shielding
Gloves
Container for Solvent Waste, 200-2,000 mL bottle
Liquid nitrogen or liquid argon
Helium 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

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Manufacture of [^{18}F]FLT Using a Nucleophilic Synthesis Module

INVENTORIED SUPPLIES (may vary depending on nucleophilic synthesis module)

Supply Description	Specification/Internal Specification Code (ITC)	Number required
Sterile Syringe	**1 mL, No silicone or latex**	8
Disposable needles	**23G or 25G X > 2 ½"**	6
Sterile Vent Filters 0.2 µM Millex SLGS V25 5F	**SS-SVF**	4
Alumina N Light Sep-Pak Cartridge	**SS-SPC**	1
[^{18}F] Separation Cartridge	**SS-FSC**	1
Sterile Disposable Syringes	**10 mL, No silicone or latex**	1
Sterile Disposable Syringes	**1-0.5 mL, 1-1mL, 1-10 mL, 2-5 mL**	5
Sterilizing Filter 0.2 µm	**SS-SPF**	2
Sterile Vented Filter Needle	**SS-VFN**	1
Empty Sterile Vial, 10 to 30 mL	**SS-SBV**	1
Sterile Disposable Needles	**22G or smaller**	8
HPLC Preparative Column (Luna C18)	**SS-LUN**	1
Alcohol swabs	**Sterile, prepackaged single swabs**	qs

REAGENTS/SOLUTIONS

Reagent	Internal Tracking Code (ITC)	Description	Quantity
Ethyl Alcohol	**SS-ETH**	USP, Absolute	qs for eluent and HPLC disinfectant and cartridge washing
SWFI, Sterile Water for Injection	**SS-WFI**	USP	qs for eluent and HPLC disinfectant and cartridge washing
Kryptofix® [2.2.2]	**SS-KRY**	≥ 97.5% Purity, off-white to white crystals or powder	15 ± 2 mg
K_2CO_3	**SS-KCA**	White granular powder	Refer to compounding record KCO-L
NaOH	**SS-NAH**	USP	Refer to compounding record NAH-SOLN-L
[^{18}F] Precursor, 5'-*O*-Benzoyl-2,3'-anhydrothymidine	**SS-BAT**	GMP grade, packed under argon atmosphere in dark glass vials; store desiccated at 4-7°C, protected from light until used.	10 ± 1 mg
Dimethyl Sulfoxide (DMSO)	**SS-DMS**	Anhydrous	1.0 ± 0.05 mL
Sodium Dihydrogen Phosphate, Monobasic	**SS-DPM**	USP	Refer to compounding record DHP-L
Acetone	**SS-ACT**	Spectrophotometric Grade	qs to clean unit
Sodium Phosphate for Injection	**SS-NAP**	USP	1 Vial

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23.4% Sodium Chloride Solution, for Injection, USP (234 mg/mL)	**SS-NCC**	USP	1 Vial
Acetonitrile	**SS-ACN**	Anhydrous	1.0 ± 0.05 mL
$H_2{}^{18}O$, Target material	**SS-O18**	≥ 80% purity	1.0 – 5.0 mL

Solutions	Specification	Compounding Code (if applicable)	Quantity
70% Vol. Ethanol	Compounded from USP reagents	**ETH70-L**	500 mL
(92/8) Sodium Phosphate 10 mM/ Ethanol	Compounded from USP reagents	**HBS-L**	> 750 mL
0.04 M K_2O_3 solution	High purity carbonate in USP water for injection	**KCO-L**	0.50 ± 0.05 mL
Kryptofix® [2.2.2] solution (15mg in 1 mL acetonitrile)	Anhydrous CH_3CN, reagent grade [2.2.2]	N/A	1.0 ± 0.05 mL
1% NaOH	Compounded from USP/NF reagents	**NAH-SOLN-L**	0.35 ± 0.05 mL
0.2N Sodium Dihydrogen Phosphate	Compounded from USP reagents	**DHP-L**	0.75 ± 0.05 mL

D. SPECIAL NOTES AND POLICIES

- Refer to the appropriate section of the Nucleophilic Synthesis Module Operator's Manual 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 Nucleophilic Synthesis Module, always use fresh, sterile syringes and needles for each prepared solution.
- To extend the lifetime of the septum seals at the reagent vials you should use **only long, thin needles that reach down to the bottom of the reagent vial.** This prevents air bubbles in the reagent vial neck; the recommendation is 23G x 22/g inch or 0.60 x 60 mm. Also additions can be made by unscrewing the caps for additions and then recapping the vials.

E. RESPONSIBILITIES

- The manufacturing site should ensure that their operator's 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]FLT-L**.

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Manufacture of [^{18}F]FLT Using a Nucleophilic Synthesis Module

I. Maintenance and Cleaning of the Nucleophilic Synthesis Module

I.1. Perform Maintenance on the Nucleophilic Synthesis Module as follows:

I.1.1. If necessary, remove columns from synthesizer, and store the used columns in a shielded waste container to decay as per the appropriate institutional radiohazardous waste policy.

I.1.2. 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.1.3. Remove the cold trap from the liquid nitrogen or argon dewar. Empty the 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.

I.1.4. Using appropriate safety gear fill the dewar halfway with new liquid nitrogen or liquid argon. Replace the dewar in the synthesizer. 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.1.5. Empty the v-vial into an approved solvent waste container. Dry the vial with a low particulate wipe and reattach it to the synthesizer.

I.1.6. Empty the waste container into an approved solvent waste container and reattach the waste container.

I.1.7. Inspect or replace the Reaction Vessel: (*Sponsor should provide these procedures here by referring to the appropriate maintenance manual for the Nucleophilic Synthesis Module.*)
I.1.7.1.
I.1.7.2.
I.1.7.3.
I.1.7.4.

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I.1.7.5.
I.1.7.6.
I.1.7.7.
I.1.7.8.

I.1.8. Record the Maintenance in the Equipment History Log Book for the Nucleophilic Synthesis Module, on **Form EQ-001**.

I.2. Cleaning the Nucleophilic Synthesis Module (the Sponsor is responsible for providing the remainder of the cleaning procedure for the corresponding synthesis module as appropriate.)

I.2.1. Turn on the compressed air and nitrogen or helium gas supplies. Verify that the gas supplies are sufficient and within at the specified pressure ranges.

I.2.2. Turn on the computer to the nucleophilic synthesis module and the power to the synthesizer's electronics unit. Initialize the module's software program.

I.2.3. The program will prompt the user to enter a password and username. Please note that all operations performed on the system should require the use of a password and username.

I.2.4.

I.2.5.

I.2.6.

I.2.7.

I.2.8.

I.2.9.

I.2.10. Document the equipment cleaning as per **GEN-R502** in the equipment history log, **Form EQ-001**.

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II. Pre-Synthesis Setup for Manufacturing [^{18}F]FLT Using Synthesis Module

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

II.1.1. Using aseptic technique, add > 200 mL of 70% ethanol, USP to the eluent bottle number 2 and 500 mL of fresh preparative mobile phase (**HBS-L**, 92% 0.01 M Phosphate 8% Ethanol) to the eluent bottle number 1.

II.1.2. On Nucleophilic Synthesis Module software go to the menu bar and select manual operation. Switch the injector to the inject position.

II.1.3. In manual operation, start the nitrogen or helium mobile phase purge gas. Check that the gas is flowing. Open valve to eluent 2, (70% Ethanol).

II.1.4. Use the keyboard to set the HPLC pump flow to 4 (mL/min). Let the HPLC pump run for at least 10 minutes. 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.

II.1.5. Set the HPLC pump flow to 0 (mL/min). Turn the valve to eluent 1, (**HBS-L**, 92% 0.01 M Phosphate 8% Ethanol).

II.1.6. Set the pump flow to 3 (mL/min). Let the HPLC pump run for at least 4 minutes. Then set the pump flow to 6 (mL/min). Let the HPLC pump run for at least 20 minutes (120 mL) to equilibrate the HPLC column. Check that the flow is steady. After 10-15 minutes measure the flow rate out of the HPLC column to assure that the system is operating correctly. (*more column rinsing will be done during synthesis).*

II.1.7. Close the valve to the He or N_2 eluent purge gas. Set the HPLC pump flow to 0 (mL/min).

II.1.8. Stop manual run and return to automatic operation.

II.2. Prepare the ^{18}F Separation Column as follows:

II.2.1. Wash the ^{18}F Separation column (**SS-FSC**) with 1 mL of ethanol, absolute, USP, (**SS-ETH**) using a 1 mL, non-lubricated syringe.

II.2.2. Wash the ^{18}F Separation column with 1 mL of sterile water for injection (**SS-WFI**) using a 1 mL, non-lubricated syringe. Wait 5 minutes.

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II.2.3. Expel the residual water from the column using 1 mL of air in the syringe. Do not dry the column any further.

II.3. Prepare the Sep-pak® Alumina N Light Cartridge as follows:

II.3.1. Wash the alumina cartridge (**SS-SPC**) slowly with 10 mL of sterile water for injection (**SS-WFI**).

II.3.2. Expel the residual liquid from the cartridge using air in the syringe.

II.4. Prepare the Synthesis Precursor Solution (5'-O-benzoyl-2,3'-anhydrothymidine, dissolved in 1 mL DMSO) as follows:

II.4.1. Draw up 1.0 ± 0.05 mL of DMSO (**SS-DMS**) using a 1.0 mL, non-lubricated syringe.

II.4.2. Add the DMSO to the vial containing 10 mg of the precursor, 5'-O-benzoyl-2,3'-anhydrothymidine (**SS-BAT**), through the septum and agitate the vial to dissolve.

II.4.3. Leave the syringe in the vial and the vial as it is until it is time to transfer the dissolved contents into the module reagent Vial 3 using the 1 mL non-lubricated syringe and long needle.

II.5. Prepare the Sterile Product Vial Assembly as Follows:

II.5.1. Label the empty product sterile vial (**SS-SBV**) with "[^{18}F]FLT" and the Master Batch Number. Swab the top and sides of the vial. Place the vial in a laminar flow hood. Reswab the top of the vial using an alcohol swab.

II.5.2. Use aseptic technique, in a laminar flow hood, to insert a sterilizing filter with a ≤ 22 G needle into the final product empty sterile vial.

II.5.3. Insert a venting filter needle into the empty sterile vial.

II.5.4. Add 0.55 to 0.60 mL of concentrated saline, for dilution, USP (**SS-NCC**) to the vial.

II.6. Run the FLT Program as follows (*make certain the target output line is connected to the Nucleophilic Synthesis Module)***:** *This section is the list of prompts for the specific computer program and the adding of reagents prior to starting the synthesis—the sponsor should provide this depending on which nucleophilic synthesis module they choose to utilize.*

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II.6.1.
II.6.2.
II.6.2.1. Prompt:
II.6.2.2. Prompt:

II.6.2.3. Prompt:
II.6.2.4. Prompt:
II.6.2.5. Prompt:
II.6.2.6. Prompt:
II.6.2.7. Prompt:

II.6.2.8. Prompt:
II.6.2.9. Prompt:
II.6.2.10. Prompt:
II.6.2.11. Prompt:

II.6.2.12. Prompt:
II.6.2.13. Prompt:
II.6.2.14. Prompt:
II.6.2.15. Prompt:
II.6.2.16. Prompt:
II.6.2.17. Prompt:

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III. Manufacturing [^{18}F]FLT Using the Nucleophilic Synthesis Module

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

III.1.1. After the requested inputs are made, press the "**START**" button to start the synthesis.

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 time that $^{18}F^-$ collection both, 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 program will take approximately 30 minutes to complete.

III.1.4. Once the synthesis is complete, the product is injected on the HPLC column (**SS-LUN**) for purification. The FLT should be visible on the Radioisotope display at approximately 16 minutes. Press the "**COLLECT**" when the FLT peak appears on the Radioisotope display. When the peak is no longer visible press the "**STOP COLLECT**" to end the collection (> 1 minute later). Record the start and stop chromatographic retention times.

III.1.5. Once the FLT program is complete remove the [^{18}F]FLT product vial from the synthesis unit.

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IV. Post Synthesis

IV.1. Withdraw Samples for Quality Control Testing

IV.1.1. Aseptically remove the sterilizing filter from the collection vial. Assay the collection vial and 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]FLT 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-001**.

IV.1.3. Detach the sterilizing filter from the delivery line, and place it in a lead shield for the filter integrity test. Perform the integrity test as per **GEN-Q110** and record the bubble point pressure on the batch record. Remove and discard the vent filters.

IV.1.4. Swab the product [^{18}F]FLT vial with an alcohol swab. Using a 0.5 or 1 mL tuberculin syringe, aseptically withdraw an accurate 0.4 mL sample for Quality Control Testing. Replace the sheath on the needle. Assay the sample and then place the syringe in a lead shield. Reswab the product vial. Transfer the shielded syringe to Quality Control for testing with **Form FQC-001**.

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 (21G or 22G) x 1½" needle on a 3-mL syringe to withdraw a 2.0 mL sample into the syringe for sterility tests (1.0 mL) and reserve sample (1.0 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.

IV.1.7. Record the results of the QC release tests on **Form FQC-001**. The tests to be conducted should include the following:

- "Radiochemical Identification & Purity of 3'-Deoxy-3'-[^{18}F]Fluorothymidine [^{18}F]FLT Injection by Radio-Thin Layer Chromatography Analysis," per SOP **GEN-Q111**

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- "Gas Chromatography Analysis of Residual Solvents," per SOP **GEN-Q112**
- "Radionuclidic Identification by Half-Life Determination," per SOP **GEN-Q113**
- "Bacterial Endotoxins by Limulus Amebocyte Lysate (LAL) Test," per SOP **GEN-Q114**
- "pH Testing of Final Product," per SOP **GEN-Q115**
- "A Color Spot Test for the Detection of Kryptofix® [2.2.2] in [^{18}F]FLT," per SOP **GEN-Q118**
- "Chemical and Radiochemical Purity by HPLC," per SOP **GEN-Q119**
- "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.

IV.1.9. Attach appropriate labels to the [^{18}F]FLT 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-R009**, "QC Testing, Review, & Final Release."

IV.1.11. Record final sterility results on **Form FQC-002** once they are complete and available. The sterility testing should be completed as per "Sterility Test," per SOP **GEN-Q117.**

V. Post-Synthesis Clean-Up of [^{18}F]FLT System (Sponsor to add additional instructions as appropriate)

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

V.1.1. Attach the lines from the system to the waste bottle.

V.1.2. Add ethanol (4 mL) to the system HPLC loading vial.

V.1.3. Change out the eluent bottle 1 containing the preparative mobile phase (HBS-L), 92% 0.01 M Phosphate and 8% ethanol to a new eluent bottle 1 containing > 200 mL of 70% ethanol.

V.1.4. Select the Nucleophilic Module's HPLC ethanol cleaning Program.

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V.1.5. Start the HPLC ethanol cleaning program.

V.1.6. Verify the cleaning program is complete.

V.1.7. Document the work on the system in the equipment history log book on **Form EQ-001**.

V.1.8. Close down the Nucleophilic Synthesis Module program.

V.1.9. Shut down the computer.

V.1.10. Shut down power to the module.

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REVISION HISTORY

Version Number	Description	Reason for Revision	Date	Author
V0	Original Record	Original record generated from revising the original copy of MPR-[^{18}F]FLT for the NCI's use, to a generic form to be used as a template.	March 1, 2005	Teresa R. Zuber

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