Form-Q320
Standard Operating Procedure of Analytical HPLC for DFO-Protein

1. **Purpose:** To describe the procedure for using HPLC in analysis the purity and concentration of reaction intermediate DFO-Protein.

2. **Scope:** The HPLC method described here will be used for determining the purity of the reaction intermediate, as well as the concentration. This result will be used for next step in the determination of protein amount use in the radiolabeling reaction.

3. **Responsibilities:** Contract Sites & [Insert your institution name here] : To follow this procedure as written and to ensure that all results, control information, and raw data are documented as specified in dedicated Quality Control Laboratory Notebooks and recorded on the appropriate testing forms.

4. **References**
   - 4.1 USP 28 ‹621› Chromatography
   - **4.2 PBS-HPLC-L,** "Master Compounding Record for Preparation of Analytical HPLC Mobile phase for ^{89}Zr-Protein"

5. **Forms**
   - **Form Q119**, "Preparation of Protein Standard Solution and Dilutions Form"
   - **Form-Q118**

6. **Policies**
   N/A

7. **Materials and Equipment**
   - **7.1.** Analytical HPLC Column Superdex TM 200 10/300GL, (GE Healthcare) or equivalent. (**SS-Super**)
   - **7.2.** High Performance Liquid Chromatography (HPLC) System consisting of a high-pressure pump, either an auto-sampler/injector, or manual injector; column; a variable or multi-wavelength UV detector, with data acquisition system.

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7.3. **PBS-HPLC-L,** "Master Compounding Record for Preparation of Analytical HPLC Mobile phase"

7.4. Automatic pipettors with disposable tips and glass graduated cylinders of appropriate sizes

7.5. Glass volumetric flasks and glass or plastic vials for dilutions

8. Procedures

8.1. The retention time for DFO-Protein is approximately 15 minutes. Sample chromatograms are shown in Figures 1. All standards must be baseline resolved (resolution > 1.5) for a valid analysis.

Parameters

UV Detector: **Set to 280 nm**
Flow Rate: **0.8 mL/min**
Eluent: **PBS-HPLC-L**
Temperature: **Room Temperature**
Column: **Superdex TM** 200 10/300GL, (**SS-Super**)

8.2. Preparation and Analysis of Sample

8.2.1. The collected solution from PD-10 column purification is diluted (1:10 v/v) with 0.9% NaCl.

8.2.2. A blank injection has been finished before the sample injection to make sure that no UV or/and radiation impurities from the column or/and the instrument.

8.2.3. Inject the sample. (This must be done in triplicate).

8.3. Data Analysis

8.3.1. Integrate all of the UV responses to obtain the peak areas for the DFO-Protein.

8.3.2. Perform the reverse correlation from the linear equation of the standards to determine the concentration of DFO-Protein in the solution.

8.3.3. Any other 280 nm UV absorbing chromatographic peaks that elute up to 40 minutes post injection, e.g. post injection and mobile phase disturbances, should be integrated individually and shown to sum to less than "5%" in the final product. Assume that the molar UV absorption coefficients for the unknown contaminants are the same as for DFO-Protein and that the DFO-Protein standard curve is applicable to the unknown contaminants.

ACCEPTANCE CRITERIA

Chemical Purity: **≥ 95%**

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