# Proteometer-UFT Kit Instructions for Use

## For mAb Ultra-Fast Titer Analysis
### Single-Pump Method (optional)

## A. General Purpose of Method

This method describes the determination of titer of therapeutic mAbs and other proteins that contain the Fc domain of human IgG. It is intended primarily to monitor mAb concentration in growth media during clone selection and product development utilizing HPLC systems equipped with a fluorescence detector.

The titer result is mAb dependent and the sample matrix may affect the peak response, therefore, it is recommended that a client-provided reference standard be utilized. However, NmAb in a buffer such as 12.5 mM L-Histidine, pH 6.0 may be used as an alternative reference standard for monitoring changes in mAb titer.

## B. Proteometer-UFT Kit Components

1. Proteometer-UFT Fluorescent Reagent (Part no. RGN-PUF-001): Store at -20 °C until use
2. Proteometer-UFT Buffer (Part no. BUF-PUF-001): Store at room temperature
3. Proteometer Reconstitution Reagent [dimethyl sulfoxide (DMSO)] (Part no. REC-PUF-001): Store at room temperature
4. Proteometer-UFT MD Unit (Part no. RCT-PUF-001)
5. Proteometer-UFT Union (Part no. ZDU-PFV-001)

## C. Required User Supplied Equipment & Reagents

1. Single pump HPLC system equipped with refrigerated autosampler, fluorescence detector, and software capable of electronic data collection and processing
   **NOTE: System must be flushed thoroughly with ultra-pure water prior to installation of Proteometer-UFT Kit (Section F.1)**
2. Client-provided mAb reference material or NIST mAb (NmAb RM 8671)
3. Clarified fermentation broth (CFB) for mAb dilution (mAb-free)
4. Vacuum source for mobile phase filtration
5. Pipet capable of dispensing 20 – 200 µL
6. Pipet capable of dispensing 100 – 1000 µL
7. 500 mL beaker
8. Ultra-pure water, 18.2 MΩ $cm^{-1}$ @ 25 °C, or equivalent
9. Acetonitrile, HPLC Grade or equivalent
10. Microcentrifuge tubes, low binding, 1.5 mL (Eppendorf Cat. No. 0030108442, or equivalent)
11. One 500 mL media bottle, low actinic
12. One 100 mL media bottle, low actinic
13. HPLC autosampler vials, or multi-well plate
14. Filtration apparatus equipped with 0.22 µm polyethersulfone (PES) membrane, or equivalent

## D. Preparation of Mobile Phase UFT-MPf-S From Supplied Components

One Proteometer-UFT Buffer packet will make 500 mL of mobile phase, which is sufficient for approximately 330 injections.

1. *Preparation of Mobile Phase UFT Component F.* Remove one vial from -20 °C freezer and allow to equilibrate to room temperature. Reconstitute Proteometer-UFT Fluorescent Reagent (Section B.1) by adding 0.40 mL Proteometer Reconstitution Reagent from container (Section B.3). Gently mix vial contents to dissolve and incubate at room temperature in the dark for at least 30 minutes. This stock solution is now Mobile Phase UFT Component F, and is usable for up to 4 weeks if stored at -15 to -20 °C.

2. *Preparation of Mobile Phase UFT-MPf-S.* Using scissors, cut open and add one packet of Proteometer-UFT Buffer (Section B.2) to approximately 400 mL ultra-pure water contained in a 500 mL beaker. Ensure quantitative transfer by rinsing out the contents

of the packet into the beaker. Stir well until dissolved. Bring the solution to a final volume of 500 mL with ultra-pure water and filter through the 0.22 µm filter into a 500 mL low actinic media bottle. Add 25 mL of acetonitrile and mix well by swirling. Quantitatively transfer contents of the vial of Mobile Phase UFT Component F (Section D.1) to the Mobile Phase UFT-MP in the low actinic media bottle by rinsing out the vial contents into the bottle using the mobile phase. Mix by swirling. This is UFT-MPf-S (treat as light-sensitive, recommended for use within 48 hours). The volume of Mobile Phase UFT-MPf-S prepared may be scaled if necessary.

## E. Sample and Standard Diluent

Mobile Phase UFT-MPf-S is also used as sample and standard diluent. About 70 mL of diluent is sufficient for approximately 330 injections. Therefore, transfer 70 mL of Mobile Phase UFT-MPf-S to a 100 mL low actinic media bottle for use as sample and standard diluent. The volume of Mobile Phase UFT-MPf-S saved for use as sample and standard diluent may be scaled as required.

## F. HPLC System Conversion – Proteometer-UFT Setup and Equilibration

The HPLC configuration for Proteometer UFT is shown in Figure 1. It is important to keep the dead volume of the system at a minimum by using the zero dead volume unions (Section B.5) provided and using minimal length of connection tubing. By minimizing the flow-path, it is ensured that the sample peak is captured within a run-time of 1.5 minutes.

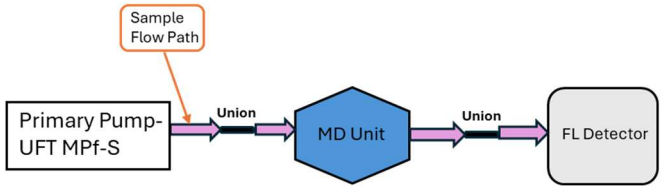


Figure 1. Proteometer-UFT Single Pump installation

1. Prior to installation of the Proteometer-UFT Kit:
   a. Install ultra-pure water vessel on the pump where Mobile Phase UFT-MPf-S will flow.
   b. Prime pump system for about 3 minutes to remove any residual contaminants and/or organic components from the system.
   c. Set the flow rate to approximately 0.3 mL/min and flush for at least 30 minutes.
2. Before installing MD unit, install Mobile Phase UFT-MPf-S on the HPLC system. Install the MD Unit (Section B.4) on the HPLC system in line with the fluorescence detector as shown in Figure 1.
3. Prime the HPLC pumps and equilibrate the HPLC system at initial method conditions of 0.6 mL/min for approximately 10 minutes.
4. Allow system to equilibrate until baseline is stable with fluorescence monitoring at Ex 552 nm /Em 578 nm. Flow rate may be initially set to 0.1 mL/min until fluorescence signal stabilizes. The complete list of instrument parameters is as follows:
   - Mobile Phase: Mobile Phase UFT-MPf-S
   - Flow Rate: 0.6 mL/min
   - Maximum Pressure of MD Unit: 1200 psi (82.7 bar)
   - Mode: Isocratic
   - Column Oven Temperature: Ambient
   - Auto-Sampler Temperature: 4°C
   - Injection Volume: 1-50 µL, constant for all samples in a run (50 µL recommended)
   - Run Time: 1.5 minutes
   - Fluorescence Wavelength Settings: Excitation 552 nm / Emission 578 nm
   - Detector Settings: Factory Default

## G. Preparation of mAb Samples, Standards, and Controls

*mAb samples*: Fermentation broth may be collected at desired time points from a mAb production run prior to, or in parallel with, the analyses. Please use the method that you would normally use to remove the cells/clarify the broth. You may store each day's CFBs in aliquots as you typically would until the day of analysis. Before transferring to an autosampler vial, centrifuge at 3000 x *g* for 30 seconds to remove any particulates.

*mAb-free CFB control*: Please note that you will also require an aliquot of mAb-free CFB as a control to monitor interference from non-mAb broth components (Blank B). mAb-free CFB may be obtained from a culture transformed with empty vector or by depleting mAb from an aliquot of the test CFB using immobilized Protein A in batch mode. Prior

to use, CFB should be centrifuged at 3000 x *g* for 30 seconds to remove any particulates.

*CFB may be viscous. Use care when pipetting CFB.*

*Samples may be injected from autosampler vials or multiwell plates. Sample and Mobile Phase UFT-MPf-S are mixed in the ratio 1:10 (v/v). The total volume of the mixture in a vial or plate well may be adjusted as desired.*

Prepare controls, standards and samples as follows:

1. *Controls*
   Blank A: 200 µL of Mobile Phase UFT-MPf-S (Section E)
   Blank B: A mixture of 20 µL of mAb-free CFB plus 200 µL Mobile Phase UFT-MPf-S
   Blank C: If the standard is prepared in buffer, a mixture of 20 µL of this buffer plus 200 µL Mobile Phase UFT-MPf-S is used as Blank C
2. *Standards*: Prepare standards at concentrations that bracket the estimated sample concentration (e.g. 0.3 to 5 mg/mL). Standard solutions should be prepared using client-provided mAb reference material (or NIST mAb) diluted with mAb-free CFB. Place a mixture of 20 µL of each standard mAb solution plus 200 µL Mobile Phase UFT-MPf-S in an autosampler vial or plate well to perform desired injections
3. *Samples*: Place a mixture of 20 µL of each test sample plus 200 µL Mobile Phase UFT-MPf-S in an autosampler vial or plate well to perform desired injections

## H. Verification of Instrumental Parameters (Fluorescence Detector Settings)

Each detector has its own strength of fluorescent signal which is dependent on factors such as instrument make and age of detector and lamp.

The same injection volume (50 µL recommended) must be used for this step and for data acquisition.

1. With the detector set to the factory default parameters, perform repeated injections of mAb in CFB (Section G.2) at the selected injection volume at the highest µg load you expect to observe in your samples (no greater than 23 µg), adjusting detector settings after each injection, until the signal is maximized without saturating the detector. This should be your detector setting for the duration of testing.

## I. Data Acquisition

At constant Injection volume (50 µL recommended),

1. Perform injections of Blank A and Blank B (or C, if applicable). Evaluate the chromatograms and if peaks are present in Blank A, take appropriate measures to clear the system of potential contaminants.
2. Generate a standard curve for titer analysis by injecting the prepared mAb standards (Section G.2) such that the amount of mAb injected is between 1.4 and 23 µg. Process the standards following J.1-J.2 to obtain peak areas. Plot the peak area versus the mAb standard concentration to determine the linear dynamic range in which all sample concentrations must fall.
3. Inject samples (Section G.3) from desired time points. Sample concentration may be adjusted by dilution with Mobile Phase UFT-MPf-S, if necessary, to fall within established linear dynamic range. Samples and blanks must be injected at the same volumes as were injected for the standards in Section I.2.

## J. Processing and Reporting

1. Evaluate chromatograms from Blank B (or C, if applicable). The area of peaks present in Blank B at these elution times must be subtracted from the corresponding sample peak area.
2. Create a processing method to consistently integrate peaks in all injections of your mAb. Elution times are dependent on the HPLC system in use.
3. Use the standard curve (Section I.2) to calculate the concentration of the mAb in the unknown fermentation samples by plotting the Peak Area versus the mAb Standard Concentration.

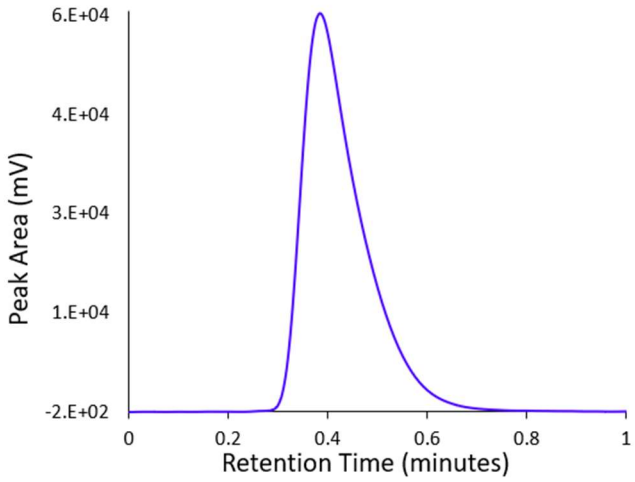


Figure 2. Example Chromatogram: NIST mAb at 23 µg load

## K. Proteometer-UFT MD Unit Extended Storage Conditions

Flush with copious amounts of ultrapure water maintained at a low flow rate to avoid excessive backpressure. Force air into the tubing with an empty syringe to displace the water, and store in a closed container.

## Ordering Information

| Description | Novilytic Part No. | U. S. Avantor Catalog No. | European Avantor Catalog No. |
|---|---|---|---|
| Proteometer-UFT Starter Kit, 300 injections (1 reagent vial, 1 buffer pack, MD unit, 1 container reconstitution reagent, tee, 2 unions) | KIT-PUF-002-01 | 77775-516 | NOVIKIT-PUF-002-01 |
| Proteometer-UFT Full Kit, 1000 injections (4 reagent vials, 4 buffer packs, MD unit, 1 container reconstitution reagent, tee, 2 unions) | KIT-PUF-010-01 | 77775-508 | NOVIKIT-PUF-010-01 |
| Proteometer-UFT MD unit | RCT-PUF-001 | 77775-502 | NOVIRCT-PUF-001 |
| Proteometer-UFT tee | TEE-PFV-001 | 77900-480 | NOVITEE-PFV-001 |
| Proteometer-UFT union | ZDV-PFV-001 | 77900-482 | NOVIZDV-PFV-001 |
| Proteometer-UFT buffer | BUF-PUF-001 | 77775-504 | NOVIBUF-PUF-001 |
| Proteometer-UFT reagent | RGN-PUF-001 | 77900-478 | NOVIRGN-PUF-001 |



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