Proteometer-L Kit Instructions for Use

For mAb Titer & Aggregate Analysis

A. General Purpose of Method

This method describes the determination of mAb titer, and relative aggregate content. It is intended primarily to monitor mAb quality during product development and production utilizing HPLC systems equipped with a fluorescence detector.

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

B. Proteometer-L Kit Components

1. Proteometer-L Reagent: Lyophilized material; each vial makes 1 L mobile phase, 14 vials per kit (store at -20°C until use) (Part # RGN-PL-001)
2. Proteometer-L Buffer: Solid material (for 1 L mobile phase); 14 packets per kit (Part # BUF-PL-001)
3. Proteometer-L Reconstitution Reagent (DMSO): 5 mL; 1 ampoule per kit (Part # REC-PL-001)
4. Proteometer-L Reactor (Part # RCT-PL-001)

C. Required User Supplied Equipment & Reagents

1. HPLC system equipped with refrigerated autosampler, fluorescence detector, and software capable of electronic data collection and processing
2. Client-provided mAb reference standard or NIST mAb (NmAb)
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. 1 L beaker
8. Ultra-pure water, 18.2 MΩ cm^{-1} @ 25 °C, or equivalent
9. Microcentrifuge tubes, low binding, 1.5 mL (Eppendorf Cat. No. 0030108442, or equivalent)
10. Media bottles, low actinic (1 L)
11. Acetonitrile, HPLC grade
12. HPLC autosampler vials, maximum recovery
13. Filtration apparatus equipped with 0.22 µm polyethersulfone (PES) membrane, or equivalent

D. Preparation of Mobile Phase L-MP From Supplied Components

1. Reconstitute one vial of Proteometer-L Reagent (Section B.1) by adding 0.320 mL of DMSO from ampoule (Section B.3). Gently mix contents to dissolve and incubate at room temperature in the dark for at least 30 minutes (stock usable for up to 1 week at 2-8 °C).

2. Add one packet of Proteometer-L Buffer (Section B.2) to approximately 800 mL ultra-pure water contained in a 1 L beaker. Ensure quantitative transfer by rinsing out the contents of the packet into the beaker. Stir well until dissolved.

3. Bring to a final volume of 1000 mL with ultra-pure water and filter through the 0.22 µm filter (Section C.13) into the low actinic media bottle (Section C.10).

4. Measure 50 mL of the HPLC grade Acetonitrile (Section C.11) and add to filtered mobile phase.

5. Quantitatively transfer the solution of reconstituted Mobile Phase L-MP Component A (Step 1) to the bottle of mobile phase prepared in Step 4 by rinsing out the contents of the Component A vial, and stopper, if necessary, into the mobile phase bottle using the mobile phase prepared in Step 4. Mix by swirling. This is Mobile Phase L-MP (recommended for use within 48 hours). Mobile phase preparation may be scaled up, if necessary.

6. Transfer sufficient volume of Mobile Phase L-MP to a maximum recovery autosampler vial (Section C.12) and place in refrigerated autosampler (Blank A).

E. HPLC System Conversion – Proteometer-L Setup and Equilibration

1. Install Mobile Phase L-MP on the HPLC system and prime. Set flow to 0.2 mL/min for 10 minutes with no reactor installed to clear lines.

2. With flow set to 0 mL/min, install the Proteometer-L Reactor (Section B.4) on HPLC system.

3. Set flow to 0.1 mL/min and increase to 1.0 mL/min in 0.1 mL/min increments (15 seconds per increment).

4. Allow system to equilibrate until baseline is stable (at least 1 hour) with fluorescence monitoring at Ex 450 nm /Em 520 nm. The complete list of instrument parameters is as follows:

   - Mobile Phase: Mobile Phase L-MP (Section D.5)
   - Flow Rate: 1 mL/min
   - Maximum Pressure of Reactor: 5800 psi (400 bar)
   - Mode: Isocratic
   - Column Oven Temperature: Ambient
   - Auto-Sampler Temperature: 4°C
   - Injection Volume: Variable
   - Run Time: 10 minutes
   - Fluorescence Wavelength Settings: Excitation 450 nm / Emission 520 nm
   - Detector Settings: Factory Default

F. Preparation of mAb Samples, Standards, and Controls

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 1000 x g for 30 seconds to remove any particulates. 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.

Every mAb sample will contain aggregates (high molecular weight species, HMW) and monomers. Percent aggregate content may vary as a function of the amount of total mAb injected (HMW plus monomer). It is therefore recommended that differences in aggregate content are monitored by injecting samples containing equivalent amounts of total mAb.

1. Samples: Prepare sufficient volume of CFB containing the test mAb. Transfer each sample to a maximum recovery autosampler vial and place in refrigerated autosampler.

2. Standard: Prepare sufficient volume of a 1 mg/mL solution of client-provided mAb by dilution with mAb-free CFB*. Centrifuge at 1000 x g for 30 seconds to remove any particulates.
   Use caution while pipetting as CFB may be viscous. Transfer aliquots of the prepared mAb standard in CFB to maximum recovery autosampler vials and place in refrigerated autosampler.

   *Note: If pure client-provided mAb and mAb-free CFB are unavailable, they may be substituted with NmAb and 12.5mM L-Histidine pH 6.0, respectively. The stock solution may be diluted to 1 mg/mL using its storage buffer.

3. Controls: Transfer sufficient volume of mAb-free CFB to a maximum recovery autosampler vial and place in refrigerated autosampler (Blank B). If the standard is prepared in buffer, transfer sufficient volume of this buffer to a maximum recovery autosampler vial and place in refrigerated autosampler (Blank C).

G. 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. Prior to testing on each system, the response of the mAb must be evaluated to ensure the appropriate linear dynamic range for your samples.

1. Perform repeated injections of mAb in CFB (Section F.2) at the highest µg load you expect to observe in your samples, 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.

H. Data Acquisition

1. For titer analysis, inject variable amounts of mAb (Section F.2) to generate a standard curve using the sum of the monomer and HMW peak areas.

2. Inject samples (Section F.1) from desired time points and adjust sample load if necessary to fall within established linear dynamic range.

3. For relative aggregate analysis, inject samples (Section F.1) from desired time points at consistent concentrations.

I. Processing and Reporting

1. Evaluate chromatograms from Blanks A and B (or C, if applicable). The profile should be absent of interfering peaks at the retention times corresponding to HMW and monomer mAb species. If peaks are present in Blank A or C, appropriate measures should be taken to clear the system of potential contaminants. Peaks present in Blank B at these retention times must be subtracted from the corresponding sample peak area. If no significant interference is observed, this control may be omitted moving forward.

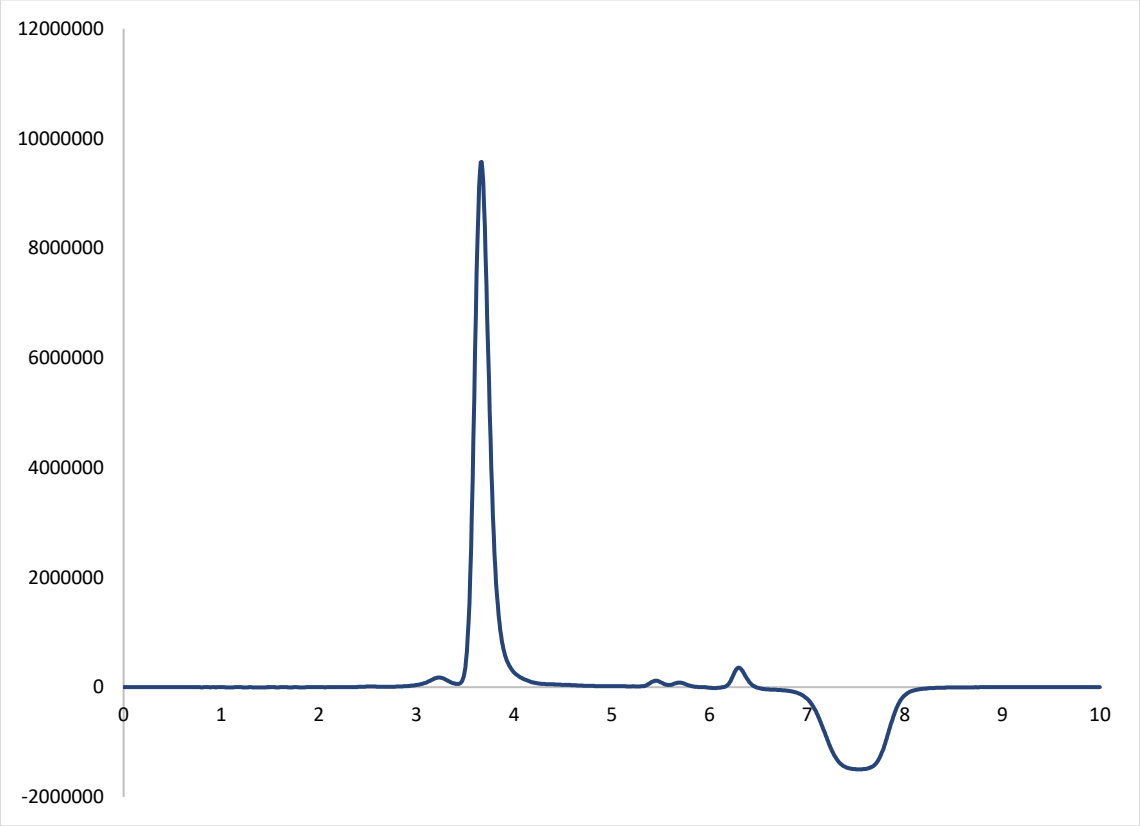
2. Create a processing method to consistently integrate HMW and monomer peaks in all injections of your mAb. Retention times are dependent on the mAb and HPLC system in use. In addition to the mAb monomer and dimer peaks, mAb trimer, tetramer, etc. peaks may also be observed. HMW peak area is reported as the sum of dimer, trimer, tetramer etc. peak areas.

3. Use the standard curve (Section H.1) to calculate the concentration of the mAb in the unknown fermentation samples

4. Calculate the aggregate content as follows:

$$\%\ \text{Aggregate content} = \frac{100 * \text{area of HMW mAb peaks}}{\text{total mAb peak area}}$$

Example Chromatogram: NIST mAb at 16µg load



J. Proteometer-L Reactor Extended Storage Conditions

100 mM sodium phosphate, pH ≤ 7, containing 0.02% sodium azide at room temperature.

Ordering Information

Description	Part Number
Proteometer-L 1-Day Kit	KIT-PL-001-001
Proteometer-L 7-Day Kit	KIT-PL-007-001
Proteometer-L 14-Day Kit	KIT-PL-014-001
Proteometer-L Reactor	RCT-PL-001
Proteometer-L Buffer	BUF-PL-001
Proteometer-L Reagent	RGN-PL-001
Proteometer-L Reconstitution Reagent (DMSO)	REC-PL-001

Novilytic, LLC
1281 Win Hentschel Blvd.
West Lafayette, IN 47906 USA
Novilytic.com
T: 1 765 631 3100 (M-F 9A-5P EST)