Frequently Asked Questions/Troubleshooting Proteometer-L Kit

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# Frequently Asked Questions

## Will the Proteometer-L Kit work for bispecific/multispecific antibodies?

Pilot studies with customers have shown successful analysis for select multispecific antibodies. Theoretically, this product should work with any Fc-based protein, although customer studies are ongoing.

## Will the Proteometer-L Kit work for on-line testing?

The kit currently supports at-line testing only. Future versions of the product may support on-line testing.

## Is the Proteometer-L Kit MS compatible?

No, the current kit is not MS compatible. Future versions of the product may support MS compatibility.

## What are the instrument requirements for the Proteometer-L Kit?

Any HPLC system equipped with a pump, autosampler, and fluorescence detector may be utilized with the kit.

## What is the linear range for the Proteometer-L Kit?

The linear range will vary based upon detector settings, lamp age, etc. The method was verified with NIST mAb 8671 and exhibited a linear response of 0.5-16 μg for titer on our HPLC systems.

## Does the mobile phase need to be filtered?

Yes, the mobile phase should be filtered before adding acetonitrile and the Proteometer-L Reagent.

## How long does the mobile phase last?

It is recommended to change the mobile phase every 48 hours for best results.



## Can I purchase individual Proteometer-L reactors and consumables for the kits?

Yes, additional consumables are available for individual sale. The product numbers may be found on our website or in the Proteometer-L Kit instructions for use.

## Will the Proteometer-L Kit work with human serum?

No. The kit is not recommended for analysis of human serum samples.

# Troubleshooting

## Column leaks

Reduce flow in 0.1 mL/min. increments to zero. Re-seat finger-tight fittings. Increase flow incrementally in 0.1 mL/min. increments to 1.0 mL/min.

## Detector signal saturation

Adjust fluorescence detector settings to reduce gain or sensitivity. Perform injections at the highest level desired. If the signal is still saturated, make further detector adjustments and injections until the monomer peak does not overload the detector.

## Split peaks

Reduce the flow in 0.1 mL/min. increments to zero. Replace the Proteometer-L reactor. Increase the flow from 0.0 mL/min. to 1.0 mL/min in 0.1mL/min increments and allow detector baseline to equilibrate for a minimum of 60 minutes, prior to making injections.

## System overpressures

Locate the origin of the pressure issues by systematically checking HPLC system components. If the Proteometer-L reactor is deemed to be the problem, clean the reactor (see Cleaning and Storage below). If this does not solve the issue, replace the reactor.

## Performance of assay suffering (tailing, poor repeatability, loss of linearity, etc.)

Replace the Proteometer-L reactor.

# Cleaning and Storage

## Cleaning Instructions

Proteometer-L Reactors may generally be usable for up to 1000 injections without the need for substantial cleaning or maintenance. However, reactor performance may decline prematurely, depending upon materials injected onto it. In cases of reduced performance, the reactor may be restored using select solutions. If suspected contaminants are strongly adsorbed, a solution of 0.5 M guanidine hydrochloride may be used to clean the reactor. If the suspected contaminants are hydrophobic in nature, solutions containing up to 50% HPLC-grade organics (i.e., EtOH, IPA, or MeOH) may be used to clean the reactor. Lastly, if caustic contaminants are suspected, dilute nitric acids (e.g., 0.1% nitric acid in 15% ACN) may be used to clean the reactor.

Choose the most appropriate buffer for your suspected contaminant, prior to trying others. Always flush the reactor in the direction of the designated flow path to prevent possible damage and maintain at a low flow rate to avoid excessive backpressure. Rinse with a minimum of five column volumes of ultra-pure water before and after flushing with the selected cleaning solution. Flush with a minimum of 20 column volumes of the cleaning solution. If contamination persists, switch cleaning solutions. If all cleaning options have been exhausted, replace the reactor.

## Short-term storage (less than two weeks)

Flush and store the Proteometer-L reactor in 100 mM Phosphate buffer, pH 7 (mobile phase is sufficient). Maintain at a low flow rate while flushing to avoid excessive reactor backpressure. Once flushing is complete, secure the end caps onto the reactor and store at room temperature.

## Extended storage (longer than two weeks)

For long-term storage, flush the Proteometer-L reactor with 10 column volumes of 100 mM sodium phosphate, pH 7, containing 0.02% sodium azide (NaN3). Maintain at a low flow rate low to avoid excessive reactor backpressure. Once flushing is complete, secure the end caps onto the reactor and store at room temperature.