

Proteograph® ONE Peptide Quantification – Quick Reference

A Set Up Labware and Reagents

1. Remove Recovery Solution and Pierce Quantitative Fluorometric Peptide Assay from 2–8 °C storage 1 hour before loading.
2. Gather required labware and reagents.
3. Load reagents as follows:
 - A3 (Standards Prep Plate)
 - Well A1 – 150 µL of Digest Standard
 - A4 (Quantification Reservoir)
 - Well 1 – 2.4 mL Peptide Assay Reagent
 - Well 10 – 3 mL Recovery Solution
 - Well 11 – 5 mL Peptide Assay Buffer
 - Well 12 – 5 mL Peptide Assay Buffer
 - B3 (300 mL Reservoir) filled with 150 mL of 70% Isopropyl alcohol

B Run Peptide Quantification

1. On the ICS Menu, select **Peptide Quantification**.
2. Load deck according to layout shown on ICS screen. Barcodes should face **RIGHT**.
3. Select **Begin Workflow**.
4. When assay run is completed, and “**Incubation Complete**” screen appears, seal Collection Plate and set aside at RT.

C Quantify Peptides

1. Read the Black Quantification Plate using a **microplate reader (Ex. 390 nm; Em. 475 nm)** and save data in valid 96-well format (.csv/.tsv/.xlsx.)
2. Click **Calculate Concentrations** in ICS and input RFU data by pasting values into ICS or uploading the file (with matching barcode) on the **Reconstitution Volume Screen**.
3. Review peptide concentration, recovery, and outliers to confirm all wells are within expected ranges.
4. Adjust reconstitution parameters, record Total Reconstitution Volume (µL), then select **Continue**.

D Dry the Peptides

1. Unseal the Collection Plate held at RT.
2. Dry peptides overnight using a vacuum concentrator.
3. Seal and store at –80°C.

Best Practices for Sample Handling

- Bring samples and reagents to room temperature before use, as chilled samples or reagents may increase RFUs.
- Select “Greiner black Plate” in the microplate reader.
- **DO NOT** dry peptides until concentration and yields are confirmed to be in expected ranges.

Proteograph® ONE Peptide Reconstitution– Quick Reference

A Set Up Labware and Reagents

1. Prepare Peptide Reconstitution Buffer using the Peptide Calibration Kit:
 - a. Thaw frozen PepCalMix.
 - b. Add 1 mL Peptide Calibration Recon Buffer to each PepCalMix tube.
 - c. Vortex 10–15 sec and centrifuge at 16,000 g for 1 min.
 - d. Combine contents of the tubes with remaining Recon Buffer using a large bottle.
 - e. Invert to mix.
2. Aliquot prepared buffer into Peptide Calibration Reconstitution Tubes at 5 mL per tube, label, and store at –80 °C until needed.
3. Before use, thaw dried peptide plate and Peptide Reconstitution Buffer; briefly sonicate and vortex buffer before use.

Best Practices for Sample Handling

- Use multiple Peptide Calibration Kits as needed to prepare enough Peptide Reconstitution Buffer for the full study. Each kit provides sufficient buffer for 4 plates.
- Avoid drying peptides **more than once**. Store at –80°C in solution if being stored after MS injection.

B Reconstitute Peptides

1. Select “**Peptide Reconstitution**” on ICS main menu.
2. Calculate required Reconstitution Buffer volume to add to Recon Buffer reservoir and use the greater of the following.
 - a. Total Required Reconstitution Volume (µL) from the Calculate Concentrations step in Peptide Quantification.
 - b. **2.5 mL** minimum (even if your calculation is lower).
3. Load labware on the work deck according to layout on screen ensuring barcodes face **RIGHT**.
4. In the **Input Fluorescence Data** section, input RFU data obtained after peptide quantification.
5. At **Peptide Reconstitution – Setup Complete**, close the front cover and run method.
6. Export data and unload Collection Plate– resulting peptides are MS-ready.
7. Seal the collection plate and store at **2–8°C** for up to 4 days or **–80 °C** for longer periods.

Support

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