

# Olink Proteomics Submission Guidelines

Comprehensive instructions for preparing, handling, and shipping samples for Olink proteomic analysis.

Thank you for choosing Creative Proteomics for your Olink proteomics analysis. This document provides comprehensive guidance on sample preparation, buffer compatibility, and shipment requirements for Olink assays. Proper handling and documentation of samples are essential to ensure the precision, reproducibility, and reliability of your protein quantification data.



## 1. Sample Collection and Preparation

#### 1.1 Serum Collection

- Collect whole blood in serum collection tubes (no anticoagulant).
- Allow blood to clot at room temperature for 15–30 minutes (do not exceed 60 minutes).
- Centrifuge at 1,000–2,000 × g for 10 minutes at 2–8 °C to separate the serum.
- Transfer the clear serum layer into clean Eppendorf tubes or 96-well plates.
- Store at -80 °C until shipment. Avoid repeated freeze-thaw cycles.

#### 1.2 Plasma Collection

- Collect whole blood in EDTA-, citrate-, or heparin-treated tubes (EDTA preferred).
- Centrifuge at 1,000-2,000 × g for 10 minutes at 2-8 °C.
- Transfer plasma carefully into clean, labeled, low-binding tubes or plates.
- Store at -80 °C immediately after aliquoting.

#### 1.3 General Sample Handling

- Minimum volume: ≥ 40 μL per sample.
- Recommended volume: 96-well plate 50 μL/sample; Eppendorf tube 100–500 μL/sample.
- Label using durable low-temperature markers or printed labels (A1, A2, A3...).
- Use protein low-binding plastics such as skirted PCR plates with −80 °C-rated sealing film.
- Ship on dry ice and ensure all samples remain frozen upon arrival.

## 2. Cell and Tissue Lysate Requirements

Sample Type	Required Total Protein Concentration	Notes
Cell lysate	0.5-1 mg/mL	Approximately 1,000 cells/μL
Tissue lysate	0.5-1 mg/mL	Ensure complete homogenization before quantification
Exosomes	0.5-1 mg/mL	Measure total protein prior to submission
Cell culture supernatant	No dilution required	Collect directly after incubation



### 3. Compatible Buffer Formulations (Brand-Free)

To avoid brand-specific endorsements, the guide lists generic buffer formulations and composition limits that have been validated in-house. If you require specific commercial product recommendations or catalog numbers, please contact our technical team to request this information privately.

#### 3.1 Recommended Cell Lysis Buffer Formulations

The following generic formulations have been validated for cell lysis and are compatible with Olink PEA assays when used as described:

- Non-ionic detergent buffer: 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40 (or equivalent non-ionic detergent), 0.1% sodium deoxycholate. Add a protease inhibitor cocktail immediately before use. Maintain final detergent concentrations ≤1%.
- Mild NP-40 style buffer: 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40, 0.1% Tween-20. Add EDTA to a final concentration <25 mM if needed. Avoid EDTA above 25 mM to prevent interference with assay chemistry.
- Gentle extraction reagent: Buffered saline with 0.1–0.5% non-ionic detergent, supplemented with protease inhibitors. Use for delicate cell types or when preserving secreted factors is required.

#### 3.2 Recommended Tissue Lysis Buffer Formulations

Generic tissue lysis formulations validated for Olink assays:

- RIPA-style buffer (gentle RIPA): 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mM EDTA. Add fresh protease inhibitors prior to use and keep samples cold during processing.
- Detergent-limited tissue buffer: 50 mM Tris-HCl (pH 7.4), 100–150 mM NaCl, 0.5% non-ionic detergent. Useful for tissue homogenates where lower detergent improves downstream detection.

**Note:** Prepare buffers fresh or store aliquots at -20 °C. Add protease inhibitors immediately before sample processing.



## 4. Buffer Preparation and Incompatibility Notes

Avoid the following conditions when preparing cell or tissue lysates for Olink assays:

- Detergent concentration > 1% (Triton, Tween).
- Ionic detergents > 0.1% (SDS, deoxycholate).
- pH outside physiological range (should remain near 7.4).
- Salt > 250 mM NaCl, > 25 mM KCl, or > 10 mM MgCl<sub>2</sub>.
- EDTA > 25 mM.
- Reducing agents (DTT) > 1 mM.
- Strong denaturants (urea > 2 M).

## 5. Cell Culture and Supernatant Collection

Procedure	Recommended Practice Purpo	se / Notes
Pre-collection incubation	Grow cells in complete medium, wash 3× with PBS, then culture 24 h in serum-free medium before collecting the supernatant.	Prevents contamination from serum-derived proteins.
Cell seeding density	Approximately $5 \times 10^3$ cells per well in 96-well flat-bottom plates.	Ensures consistent cell density and protein levels.

# 6. Sample Submission and Shipping

- · Submit samples in multiples of 88 to align with Olink plate capacity.
- Standard samples (serum and plasma) can be submitted directly without pre-testing.
- Non-standard samples (cell lysate, tissue homogenate, exosomes) should include 3–4 pilot samples (50–60  $\mu$ L each, 0.5–1  $\mu$ g/ $\mu$ L protein).
- · Label all tubes or wells with project ID, sample ID, and sample type.
- Store at -80 °C until shipment.
- Ship on dry ice; samples must remain frozen throughout transport.
- Include a complete sample manifest listing all IDs, matrices, and concentrations.

# 7. Contact and Support



<u>Creative Proteomics - Olink Proteomics Service Team</u>



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