Olink®

Running alternative matrices

Buffer Compatibility with Olink

Buffers can be used for preparation of cell and tissue lysates, elution of proteins from solid supports, and lavage solutions. The main considerations for buffer formulation are: i) proteins should be maintained under native conditions for proper antibody recognition, and ii) be careful of reagents that can interfere with PCR.

Recommendations for Buffer Formulation

Detergents

- Excessive detergent concentration can lead to protein denaturation
- The concentration of ionic detergents should be ≤0.1%, including sodium dodecyl sulfate (SDS) and deoxycholic acid (DCA; also known as deoxycholate)
- Non-ionic detergent concentrations, such as Tween® 20, Triton™ X100, and NP-40 should be ≤1%

Other denaturants to avoid

- Dithiothreitol (DTT) in excess of 1 mM
- Alcohol
- Heat
- Urea
- Heavy metal salts

Salts

- High salt concentrations can promote the aggregation and precipitation of proteins
- Concentrations of common salts should be: ≤250 mM NaCl, ≤25 mM KCl, and ≤10 mM $MgCl_2$

pH

- The pH of buffers should be close to physiological levels (7-8 range)

Commercial buffers

- T-PER™ Tissue Protein Extraction Reagent (#78510) and M-PER™ Mammalian Protein Extraction Reagent (#78501) from Thermo Fisher are recommended.
- Bio-Plex® Cell Lysis Kit (Bio-Rad #171304011). Add the following to the buffer just prior to use: Factors 1 and 2 (from the kit) + protease inhibitor cocktail.
- RIPA buffer can be custom made as: 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X100, 0.1% DCA. We have tested commercial brands, and Millipore RIPA Lysis Buffer (#20-188) works well with Olink technology, whereas Sigma RIPA Buffer (#R0278) does not.
- NP40 lysis buffer can be custom made as: 1X Tris-EDTA buffer (pH 8.0), 1% NP-40, 0.1% Triton X100, 0.1% sulfobetaine, 150 mM NaCl, and 1X protease inhibitor cocktail.

Diluents

- The original lysis/elution buffer should be used when normalizing samples to a set protein concentration
- Olink Diluent should be used when Olink panels or alternative matrices require pre-dilution of samples

PCR inhibitors

- EDTA should be ≤25 mM to avoid sequestration of Mg^{2+}
- Urea (found in urine; pre-dilute samples at least 1:4 with Olink Diluent)
- Heme (found in hemolysates; refer to validation documents for acceptable limits)

Fluorescent dyes

- When running samples on our Target platform, be careful of the presence of fluorescent dyes that are known to interfere with quantitative PCR, such as fluorescein

Protease and phosphatase inhibitors

- It is generally recommended to include protease inhibitors within cell and tissue lysis buffers
- Roche cOmplete™ Mini Protease Inhibitor Cocktail (#11836153001) is highly recommended. One tablet can be dissolved in 10 ml of lysis buffer. Alternatively, a 10X solution can be prepared by dissolving 1 tablet in 1 ml of distilled water or PBS, or a 7X stock in 1.5 ml. The stock solution can be stored at 4°C for ≤2 weeks or -20°C for ≤12 weeks. Use a 1X final concentration of inhibitor cocktail and avoid excess final concentrations (e.g., 2X or 3X).
- General concentrations of protease inhibitors known to be compatible are: 1 mM PMSF, 10 mM AEBSF, 8 μM aprotinin, 0.2 mM leupeptin, 0.4 mM bestatin, 0.15 mM pepstatin, and 0.15 mM E-64
- Olink assays do not recognize phosphorylation sites, but phosphatase inhibitors such as NaF in the range of 5-10 mM and Na_3VO_4 at 1-2 mM are acceptable, as well as cocktails such as PhosSTOP™

Multi-omic studies

- Protein samples for multi-omics studies should be processed separately since many protocols for RNA/DNA isolation contain strong denaturants such as phenol and/or guanidinium thiocyanate
- PAXgene RNA® tubes, Allprotect® Tissue Reagent, and RNAlater® are not compatible with Olink
- Cell-Free DNA BCT® tubes from Streck are compatible with Olink

Please contact support@olink.com for further information on running alternative matrices.

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