

Running alternative matrices

Analysis of Conditioned Media

Study Design Considerations

Protein assays within Olink panels have been optimized for the dynamic range present in human plasma and serum. Results are reported as NPX™ units which are used to compare relative changes in protein abundance between study groups. Identification of true biological differences between study groups is facilitated by reducing technical variability to the fullest extent possible. This includes using the same collection procedure for each sample, keeping the same number of freeze/thaw cycles, and maintaining even storage conditions.

Within a particular study, all samples should be randomized across all plates, and it is best to use a balanced number of samples across the study groups.

In addition to plasma and serum, strategies have been developed to analyze alternative types of samples. Conditioned media, also known as cell culture supernatants, refers to the media that has been used to nourish cells grown *in vitro*. This media is of interest to researchers since it contains secreted proteins. Cultured cells can be homogenous, such as clonal primary cells and immortalized cell lines, or heterogenous such as organoids or *ex vivo* biopsies.

Samples should be normalized by volume. To evaluate protein assays at risk for hook, it is recommended to run a few samples from each study group at two additional dilutions. This is of particular importance when you expect that certain proteins of interest are present at very high abundance, such as induced cytokines and chemokines.

To maximize protein concentration of conditioned media samples, it is better to increase cell number and reduce media volume as much as possible.

Additional recommendations are that biological replicates should be included to account for technical differences in sample preparation, and complete media alone sample(s) must be included to monitor background noise contributed by media. Technical replicates can also be included for better estimation of CVs when using an alternative matrix.

Special attention should be paid to the media formulation as described below.

Recommendations for Sample Preparation

- Some media formulations contain growth serum, such as fetal bovine serum (FBS) or fetal calf serum (FCS), as an additive. Olink recommends using heat-inactivated serum to limit potential cross-reactivity of proteins that are closely homologous to human proteins.

Note: Another option would be to wash cells 3X with PBS, and then culturing for 24 h in serum-free media prior to harvesting samples. The health of cells under these conditions should be monitored in preliminary experiments, and it may be necessary to use timepoints that are <24 h.

Note: When including serum in media formulations it is important to include the appropriate controls (complete media alone) to assess background levels.

- After carefully collecting conditioned media from cells, high speed centrifugation at 4°C should be used to remove cells, cell debris, and other particulates.
- Samples should be aliquoted and stored at -80°C.
- Phenol red is known to interfere with one of the internal incubation controls for the Target 96 assay, therefore this should be taken into consideration when performing QC analysis. Target 48, Flex, Focus, and Explore are not affected.

Dilution Strategies

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1

Target 48:

1:1

Note: Dilutions are denoted as A:B, where A=number of sample units and B=total number of units after dilution, therefore 1:1 = undiluted or 'neat' sample.

Publications using Olink

Vondra S, et al. The human placenta shapes the phenotype of decidual macrophages. *Cell Rep.* 2023; 42(3):112285. DOI: 10.1016/j.celrep.2023.112285. [Link](#) [decidual explants]

Gao Y, et al. Immunodeficiency syndromes differentially impact the functional profile of SARS-CoV-2-specific T cells elicited by mRNA vaccination. *Immunity.* 2022; S1074-7613(22)00338-7. DOI: 10.1016/j.immuni.2022.07.005. [Link](#) [peripheral blood mononuclear cells]

Rogic A, et al. High endogenous CCL2 expression promotes the aggressive phenotype of human inflammatory breast cancer. *Nat Commun.* 2021; 12(1):6889. DOI: 10.1038/s41467-021-27108-8. [Link](#) [breast cancer cell lines]

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