

Analysis of Amniotic Fluid

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 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. Amniotic fluid is a clear, yellowish fluid found within the amniotic sac which surrounds the fetus during pregnancy. At first, amniotic fluid is mainly water with electrolytes, but by about week 13 the liquid also contains proteins, carbohydrates, lipids, and urea which aid in growth. Sampling of amniotic fluid can impart information on the health and development of the fetus.

Samples are 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. It is not necessary to include biological replicates or to add protease inhibitors. Technical replicates can be included for better estimation of CVs when using an alternative matrix.

Recommendations for Sample Preparation

Sample collection

- Amniotic fluid should be collected using best practice clinical guidelines.
- Freshly collected samples are stable for a short duration at room temperature but should be stored on ice or at 4°C if possible.
- Samples should be centrifuged for 10 min at $\geq 500 \times g$ to remove cells and insoluble material.
- Samples contaminated with blood should be avoided.
- Aliquots should be stored at -80°C.

Pre-Dilution Strategies

Target 96:

CAM	CRE	CVDII	CVDIII	DEV	IMO	INF	IRE	MET	NEU	NEX	ODA	ONCII	ONCIII
1:100	1:1	1:1	1:10	1:10	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.

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

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