

# Analysis of Bone Marrow

## Study Design Considerations

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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. Bone marrow is the spongy tissue inside of larger bones. Bone marrow has a fluid portion, which can be sampled by aspiration, and a solid portion which can be sampled by biopsy. Bone marrow can be used to investigate anemia, blood and metastatic cancers, and other diseases and infections.

Olink recommendations for *Kit Users Running Alternative Matrices: Analysis of Cell Culture Lysates* should be followed when isolating specific cell types within bone marrow. The guidelines below refer to the clarified liquid fraction of bone marrow (alternatively referred to as supernatants or plasma). 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

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### Sample collection

- Bone marrow should be collected using best practice clinical guidelines.
- A protocol for bone marrow aspiration from the posterior superior iliac spine has been outlined by Cloos et al. (*J Vis Exp.* 2018; 133: e56386, DOI: 10.3791/56386) and considerations include:
  - Spicules (small bone fragments) indicate that the bone marrow cavity has been accessed and that aspiration has been performed correctly
  - Most spicules will be in the first 1-2 ml from the initial withdrawal
  - Aspirate only 1-2 ml to avoid diluting the sample (hemodilution could be a confounding factor in proteomic analysis)
  - In the case of slow or difficult bone marrow collection, use of syringes pre-flushed with anticoagulant may be helpful
- 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 2000 x g to remove cells and insoluble material.
- Aliquots should be stored at -80°C.

## Pre-Dilution Strategies

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### 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.

## Publications using Olink

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Wong S, et al. Multi-omic analysis of the tumor microenvironment shows clinical correlations in Ph1 study of atezolizumab +/- SoC in MM. *Front Immunol.* 2023; 14:1085893. DOI: 10.3389/fimmu.2023.1085893. [Link](#)

Weivoda MM, et al. Identification of osteoclast-osteoblast coupling factors in humans reveals links between bone and energy metabolism. *Nat Commun.* 2020; 11(1):87. DOI: 10.1038/s41467-019-14003-6. [Link](#)

Ji J, et al. Targeting HMGB1 by ethyl pyruvate ameliorates systemic lupus erythematosus and reverses the senescent phenotype of bone marrow-mesenchymal stem cells. *Aging (Albany NY).* 2019; 11(13):4338-53. DOI: 10.18632/aging.102052. [Link](#)

Please contact [support@olink.com](mailto:support@olink.com) for further information on running alternative matrices.

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