Human B-cell assay sample preparation guidelines

Profiling resolutions: survey vs. deep

Sample type, the number of expected B cells and the experimental question are important factors to consider when deciding on a profiling resolution.

RESOLUTION	CONSIDERATIONS
Survey	• Clonal samples • Samples with low numbers of B cells (≤ 100,000 cells) • Samples derived from most non-lymphoid tissues
Deep	• Studying the peripheral immune repertoire (e.g., peripheral blood mononuclear cells [PBMCs] or lymphoid tissue) • Samples requiring greater sensitivity (detection of rare clones) • Experiments assessing a broader range of the repertoire • Samples with 100,000–200,000 B cells

Target quantities for extracted gDNA

SAMPLE TYPE (TARGET MASS OR CONCENTRATION)	IGH ASSAY	
	Survey (in 50 µL TE)	**Deep (in 100 µL TE)**
Sorted cells	20 ng/µL; 1 µg	30 ng/µL; 3 µg
Peripheral blood mononuclear cells (PBMCs)	200 ng/µL; 10 µg	300 ng/µL; 30 µg
Bone marrow aspirate	200 ng/µL; 10 µg	300 ng/µL; 30 µg
Bone marrow mononuclear cells (BMMCs)	60 ng/µL; 3 µg	90 ng/µL; 9 µg
Lymphoid tissue	34 ng/µL; 1.7 µg	50 ng/µL; 5 µg
Non-lymphoid tissue	60 ng/µL; 3 µg	90 ng/µL; 9 µg
Formalin-fixed, paraffin embedded tissue (FFPE)	25 microns in 50 µL (maximum scroll thickness of 10 microns each)	50 microns in 100 µL (maximum scroll thickness of 10 microns each)
Bone marrow aspirate smear slide	25 microns in 50 µL (maximum scroll thickness of 10 microns each)	50 microns in 100 µL (maximum scroll thickness of 10 microns each)

Work with archived, fresh or frozen lymphocyte containing samples

ASSAY AVAILABLE

Immunoglobulin heavy chain (IGH)

SHIPPING

We will send you a return shipping box containing labware and sample shipping instructions. Please use the materials provided to send your samples.

FOR QUESTIONS, CONTACT

customercare@adaptivebiotech.com
Academic research customers

bdclientmanagers@adaptivebiotech.com
Pharma and biotech customers

Note: Elute to the requested volume, independent of concentration.

- For cDNA samples, we recommend starting with a minimum of 150 ng of total RNA.
- Due to low composition of B cells in whole blood, we do not recommend using whole blood as a sample type for most B-cell projects.
- For tissue samples at survey resolution, we request a minimum of 400 ng of gDNA.

Considerations for extracting gDNA

- In order to reach the amount of genomic DNA (gDNA) and concentrations outlined above—and to accurately estimate the input requirement—it is critical to know the extraction efficiency of your extraction method.
- The minimum number of input B cells is 1,000.
- Adaptive Immunosequencing is compatible with less gDNA than outlined in the chart above; however, submitting gDNA at a concentration less than 10 ng/µL limits our ability to troubleshoot issues.

* "Survey" and "deep" are terms to represent the breadth of the immune repertoire assayed (i.e., number of B cells). The terms are not indicative of the data quality.

Recommendations for extracting gDNA

ISOLATING DNA FROM DIFFERENT SAMPLE TYPES

Sorted cells

- We recommend sorting cells into HEPES buffer (PBS with 2% FBS and 0.025 M HEPES) to boost the gDNA yield from cell pellets.
- When preparing fixed cells for fluorescence-activated cell sorting (FACS), a concentration of 0.5%–2% paraformaldehyde (PFA) is recommended; higher concentrations of PFA can fragment the gDNA, which will result in reduced PCR amplification efficiency.

Tissue

- A tissue homogenizer with homogenization buffer is recommended for disruption of fresh or frozen tissue samples.

Formalin-fixed, paraffin-embedded (FFPE)

- Due to cross-linking of nucleic acids and increased gDNA shearing, FFPE samples may yield low quantities of viable gDNA for immunosequencing.
- Many factors can impact the yield and quality of FFPE-derived gDNA. For best results we recommend using:
  - Buffered fixatives
  - Low fixative concentration
  - Short fixation time
  - Limited storage time

Blood, PBMCs or bone marrow

- EDTA is recommended as an anticoagulant for whole blood or bone marrow collection; however, excessive amounts of EDTA can inhibit PCR.
- Sodium heparin and sodium citrate are compatible with the Immunosequencing assay; however, excessive amounts of sodium heparin can inhibit PCR.
- Approximately 50% of cells frozen in dimethyl sulfoxide (DMSO) will lyse during the thawing process; for best results, extract gDNA from the entire thawed sample without centrifuging.

QUALITY OF GDNA

Once DNA is isolated, quantification using a spectrophotometer or comparable method is highly recommended. For optimal results, the absorbance ratios of DNA samples should be:

- A260/280 = 1.8–2
- A260/230 = 2–2.2

Coverage

- Sequencing coverage is assay-dependent.
- For cDNA, we do not target a minimum coverage. Clonality and quantification of templates does not apply for cDNA; rather, template frequency reflects relative expression levels of receptors.

POTENTIAL PCR INHIBITORS

Sample source(s) containing any of the following may inhibit PCR steps used in the Immunosequencing assay:

- **Heparin**, **EDTA**: common anticoagulants in blood and bone marrow samples
- **Melanin**: common to skin and melanoma tissue samples
- **B5 reagent**: commonly used for bone marrow storage
- **Collagen**: can be at high levels in some tissue samples
- **Myoglobin**: common to muscle tissue
- **Bacterial contamination** from all sample sources
- **Phenol**, **ethanol**, and **other organic contaminants** remaining after DNA extraction

EXTRACTION KITS

Any validated gDNA extraction method may be used to prepare sample gDNA for Immunosequencing. We do not exclusively recommend or provide technical support for any of the gDNA extraction products named here. Contact the kit manufacturer with questions or for technical support.

Example extraction kits

- QIAGEN DNeasy Blood & Tissue Kit (Mini Spin Columns)
- QIAGEN QIAamp DNA Blood Maxi or Micro Kit

For FFPE samples

- QIAGEN Deparaffinization Solution
- QIAGEN QIAamp DNA FFPE Tissue Kit

For questions, contact: **Academic research customers** customercare@adaptivebiotech.com | **Pharma and biotech customers** bdclientmanagers@adaptivebiotech.com