Mouse Sample Preparation Guidelines

Sample Types Accepted

- Sorted T and B cells
- Whole blood
- Tissue (including Fresh Frozen [FF])
- gDNA and cDNA*

Assays available:

- T-cell receptor beta (TCRB)
- IGH (assay is run on a quarterly basis)

Coverage:

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

*cDNA is not currently supported for the mouse IGH assay.

NOTES:

- Please elute to the requested volume, independent of concentration
- For cDNA samples we recommend starting with a minimum of 150 ng of total RNA
- Deep resolution is recommended for lymphoid tissue samples. For tissue samples at Survey resolution, we request an absolute minimum of 400 ng of gDNA

PROFILING RESOLUTIONS: SURVEY VS. DEEP

Sample type, the number of expected T or B cells, and the experimental question you are trying to answer are important factors to consider when deciding on profiling resolution.

Resolution	Considerations for choosing resolution
Survey[1]	• Clonal samples • Samples with low numbers of T or B cells (≤ 100,000 cells) • Samples derived from most non-lymphoid tissues
Deep[1]	• Samples requiring greater sensitivity (detection of rare clones) • Experiments assessing a broader range of the repertoire • Samples with 100,000-200,000 T- or B-cells

1. Survey and Deep are names to represent the breadth of the immune repertoire assayed (e.g. number of T or B cells); the names do not represent the quality of data or results generated

IMMUNOSEQ ASSAY SAMPLE GUIDELINES

Sample type (Target Mass or Concentration)	Profiling Resolution	
	Survey (in 50µL TE)	Deep (in 100µL TE)
Sorted cells	0.5 µg DNA 10 ng/µL	1.5 µg DNA 15 ng/µL
Whole blood	100 µL blood 0.6 µg DNA 12 ng/µL	200 µL blood 1.8 µg DNA 18 ng/µL
Lymphoid tissue	1 µg DNA 20 ng/µL	3 µg DNA 30 ng/µL
Non-lymphoid tissue	3 µg DNA 60 ng/µL	—

CONSIDERATIONS FOR EXTRACTING GDNA

- It's critical to know the extraction efficiency of your extraction method to accurately estimate the input requirement in order to reach the amounts of gDNA and concentrations outlined in the chart above
- The minimum number of input T or B cells is 1,000
- immunoSEQ Assays are compatible with less gDNA than outlined in the chart above; however, submitting gDNA at a concentration less than 10 ng/uL limits our ability to troubleshoot issues

RECOMMENDATIONS FOR EXTRACTING GDNA

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%-2.0% paraformaldehyde (PFA) is recommended. Higher concentrations of PFA can fragment the DNA, 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

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 immunoSEQ Assay. However, excessive amounts of sodium heparin can inhibit PCR
- Roughly 50% of cells frozen in DMSO will lyse during the thawing process; for best results, extract gDNA from the entire thawed sample without centrifuging

Extraction Kits

Any validated DNA extraction method may be used to prepare sample DNA for the immunoSEQ Assay. We do not exclusively recommend or provide technical support for any of the DNA extraction products named. Please contact the kit manufacturer with questions or for technical support.

Example extraction kits:

- Qiagen DNeasy® Blood & Tissue Kit (Mini Spin Columns)
- QIAamp® DNA Micro Kit

ADDITIONAL CONSIDERATIONS

Quality of Genomic DNA (gDNA)

Isolated gDNA should be quantified using a spectrophotometer or comparable method. Optimal quality of gDNA should have absorbance ratios:

- A260/280 = 1.8–2.0
- A260/230 = 2.0–2.2

Potential PCR inhibitors

Sample source(s) containing any of the following may inhibit PCR amplification:

- **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

For questions or Technical Support contact:
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