Mouse Sample Preparation Guidelines

PROFILING RESOLUTIONS: SURVEY VS. DEEP

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

Resolution	Considerations for choosing resolution
Survey	• Clonal samples • Samples with low numbers of T or B cells (≤ 100,000 cells) • Samples derived from most non-lymphoid tissues
Deep	• Studying the peripheral immune repertoire (e.g., whole blood, 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 T- or B-cells

NOTE: Please refer to our Experimental Design Reference Guide for more details (immunoSEQ.com/knowledge-center).

Assays available:

• T-cell receptor beta (TCRB)

Shipping:

When you place an order we will send you a return shipping box containing lab-ware and instructions. Please use provided materials to send your samples.

For questions, please contact:

customercare@adaptivebiotech.com
Academic Research customers

busdev@adaptivebiotech.com
Pharma & Biotech customers

TARGET QUANTITIES FOR EXTRACTED gDNA

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	0.6 µg DNA 12 ng/µL	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	—

NOTE:

- Please elute to the requested volume, independent of concentration.
- immunoSEQ Assays are compatible with less gDNA than outlined; however, submitting gDNA at a concentration less than 10ng/µL limits our ability to troubleshoot issues
- The minimum number of input T cells is 1,000. For more information see our Service Sample FAQs (immunoseq.com/knowledge-center/immunoseq-assays)
- For cDNA samples we recommend starting with a minimum of 150 ng of total RNA

TARGET STARTING QUANTITIES FOR EXTRACTING DNA

Sample type	Profiling Resolution	
	Survey	Deep
Sorted cells	3,000 – 100,000 cells	100,000 – 200,000 cells
Whole blood	50 – 200 µL	> 200 µL
Lymphoid tissue	5 – 10 mg	10 – 20 mg
Non-lymphoid tissue	5 – 10 mg	10 – 20 mg

RECOMMENDATIONS FOR EXTRACTING DNA

Sorted cells

- We recommend sorting cells into HEPES buffer (PBS with 2% FBS and 0.025M HEPES) to boost the DNA 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
- Cells should arrive in no more than 200 µL of buffer

Tissue

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

Blood

- EDTA is recommended as an anticoagulant for whole blood collection
- While sodium heparin and sodium citrate have been compatible with the immunoSEQ Assay, 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 DNA 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

Coverage

- For gDNA an average of 10X sequencing coverage is targeted across templates in a sample. However, coverage is variable depending on the number of input templates per sample.
- 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.

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 Services contact:
techsupport@adaptivebiotech.com or (855) 466-8667

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