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:

- 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

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

NOTES:

- 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
- 1,000 cells is the absolute minimum number of B or T cells accepted
- For cDNA samples we recommend starting with a minimum of 150 ng of total RNA. cDNA is not supported for the mouse IGH assay
- Deep resolution is recommended for lymphoid tissue samples

TCRB IMMUNOSEQ ASSAY SAMPLE GUIDELINES

Sample type (Target Mass or Concentration)	Profiling Resolution	
	Survey (in 50µL TE)	Deep (in 100µL TE)
Sorted cells	60,000 cells 0.5 µg DNA 10 ng/µL	200,000 cells 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	10 mm FF tissue 1 µg DNA 20 ng/µL	3 µg DNA 30 ng/µL
Non-lymphoid tissue	10 mm FF tissue 3 µg DNA 60 ng/µL	—

IGH IMMUNOSEQ ASSAY SAMPLE GUIDELINES

Sample type	Resolution	
	Survey	Deep
Sorted B cells	60,000 cells 0.5 µg DNA 50 µL vol.	200,000 cells 1.5 µg DNA 125 µL vol.
Lymphoid tissue	10 mg FF tissue 1 µg DNA	3 µg DNA
Non-lymphoid tissue	10 mg FF tissue 3 µg DNA	–

DESCRIPTION OF PROFILING RESOLUTIONS: SURVEY VS. DEEP

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

RECOMMENDATIONS FOR SAMPLE PREPARATION

Isolating DNA from different sample types

Sorted cells

- Sorting fixed cells into HEPES (PBS with 2% FBS and 0.025M HEPES) buffer can boost the DNA yield from the 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, PBMCs, or bone marrow

- ACD or EDTA is recommended as an anticoagulant for whole blood or bone marrow collection
- 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. To recover all DNA, do not centrifuge the sample after thawing. Instead, extract DNA from the entire thawed sample

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

Shipping Samples

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

For questions, please contact: customercare@adaptivebiotech.com

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 steps used in the immunoSEQ 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

For questions or Technical Support contact:
techsupport@adaptivebiotech.com or (855) 466-8667

For Research Use Only. Not for use in diagnostic procedures.

IMMUNOSEQ, AMPLIFY DISCOVERY, ADAPTIVE BIOTECHNOLOGIES and associated designs are trademarks of Adaptive Biotechnologies.

Copyright © 2018 Adaptive Biotechnologies Corp. All rights reserved.
MRK-00073-5