Human Sample Preparation Guidelines

Compatible with FFPE tissue derived DNA!

Acceptable Sample Types:

- Sorted or stimulated T cells
- Peripheral blood mononuclear cells (PBMCs)
- Whole blood
- Bone marrow
- Bone marrow mononuclear cells (BMMCs)
- Lymphoid and non-lymphoid tissue
- gDNA from T-cell containing samples

Recommended Input DNA Quantities

Determining the quantity of DNA that is needed from different sample types for the immunoSEQ Assay depends on two main criteria:

- Number of T cells being assayed
- Percentage of T cells in the sample

The number of T cells being assayed will determine the number of PCR reactions (resolution) that should be performed per sample.

DESCRIPTION OF PROFILING RESOLUTIONS: SURVEY VS. DEEP

Resolution	Number of reactions	Considerations for choosing resolution
Survey	2 reactions	• Clonal samples • Samples with low numbers of T cells • Samples derived from most non-lymphoid tissues
Deep	4 reactions	• Studying the peripheral immune repertoire (e.g., samples from whole blood, PBMCs, or lymphoid tissue) • Samples requiring greater sensitivity (detection of rare clones) • Experiments assessing a broader range of the T-cell repertoire

NOTE: immunoSEQ Kits can be used to run the exact number of reactions that best supports an experimental design, but fewer than two reactions per sample is not recommended or supported.

The table below provides expected T-cell range for several common sample types along with desired DNA concentration ranges and estimates for the total amount of DNA needed for a Survey and Deep resolution.

Other resolutions (eg Ultra Deep) can be run by using the recommended DNA concentrations over more replicates.

DNA INPUT RECOMMENDATIONS FOR DIFFERENT SAMPLE TYPES

Sample Type	Typical range of T-cell content	Desired range of gDNA concentrations for survey (ng/µL)[a]	Total gDNA for Survey (ng) (2 replicates)[b]	Desired range of gDNA concentrations for Deep (ng/µL)[a]	Total gDNA for Deep (ng) (4 replicates)[b]
Sorted T cells	80–95%	11–14	400–500	17–21	1,200–1,500
PBMCs	30–40%	27–36	1,100–1,600	41–54	3,380–4,700
Whole blood	15–25%	44–72	1,590–2,600	65–108	4,680–7,800
Bone marrow	5–15%	83–250	2,700–9,000	N/A	N/A
BMMCs	15–25%	44–72	1,590–2,600	65–108	4,680–7,800
Lymphoid tissue	40–60%	21–31	650–1,000	27–40	1,900–3,000
Non-lymphoid tissue	1–15%	83–300[c]	2,700–12,000[c]	N/A	N/A
FFPE tissue	Variable	Extract DNA from at least 25 µm in 50-100 µl and use 2+ reactions as needed			
cDNA	Please contact us at techsupport@adaptivebiotech.com for more information				

[a] Based on using 18 µL of DNA per replicate at listed concentration in 50-100 µL first PCR.

[b] Based on recommended starting input of 30,000-45,000 T-cell genomes per reaction; DNA quantification by absorbance at 260/280 nm; each genome contributes ~6.6 pg of DNA.

[c] Does not achieve 30,000-45,000 input T cells.

N/A = not applicable

RECOMMENDATIONS FOR SAMPLE PREPARATION

Isolating DNA from different sample types

Sorted Cells

- 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
- Sorting fixed cells into HEPES buffer (PBS with 2% FBS and 0.025M HEPES) can boost the DNA yield from the cell pellets

Tissue

- A tissue homogenizer with homogenization buffer is recommended for disruption of fresh or frozen tissue samples
- Possible extraction kits:
  - QIAGEN DNeasy® Blood & Tissue Kit (Mini Spin Columns)

Blood, PBMCs, or bone marrow

- EDTA is recommended as an anticoagulant for whole blood or bone marrow 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. To recover all DNA, do not centrifuge the sample after thawing. Instead, extract DNA from the entire thawed sample
- Possible extraction kits:
  - QIAGEN DNeasy® Blood & Tissue Kit (Mini Spin Columns)
  - QIAGEN QIAamp® DNA Blood Maxi Kit

NOTE: Any validated DNA extraction method may be used to prepare sample DNA for the immunoSEQ Assay. Adaptive Biotechnologies does not exclusively recommend or provide technical support for any of the DNA extraction products named. Please contact the extraction kit manufacturer for any questions or technical services.

Quality of input DNA

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 = 1.8–2.0
- 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 Services contact:
techsupport@adaptivebiotech.com

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MRK-00020-5