Human Sample Preparation Guidelines

Appropriate Sample Types:

- Sorted T cells
- Peripheral blood mononucleated cells (PBMCs)
- Whole blood
- Bone marrow
- Bone marrow mononuclear cells (BMMCs)
- Lymphoid and non-lymphoid tissue

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-cell content of the sample

The number of T cells being assayed will determine the number of PCR reactions (resolution) that should be performed per sample. The table to the right outlines two resolutions (Survey and Deep) recommended for new users of the immunoSEQ Human T-cell Receptor Beta (hsTCRB) Kit.

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	6 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.

Recommended quantities of total input DNA (non-T-cell + germline T-cell + rearranged T-cell DNA) for Survey and Deep assays are outlined in the table below. Input amounts are based on sample type.

DNA INPUT RECOMMENDATIONS FOR DIFFERENT SAMPLE TYPES

Sample type	Typical range of T-cell content	Desired range of DNA concentrations (ng/µL)[a]	Total gDNA for Survey (ng) (2 replicates)[b]	Total gDNA for Deep (ng) (6 replicates)[b]
Sorted T cells	>90%	12–14	400–450	1,200–1,350
PBMCs	40–70%	18–31	600–1,000	1,800–3,000
Whole blood	20–40%	31–62	1,000–2,000	3,000–6,000
Bone marrow	5–15%	83–250	2,700–8,000	N/A
BMMCs	20–40%	31–62	1,000–2,000	3,000–6,000
Lymphoid tissue	40–60%	21–31	700–1,000	2,100–3,000
Non-lymphoid tissue	1–15%	83–300[c]	2,700–10,000[c]	N/A

[a] Based on using 16 µL of DNA at listed concentration in 50 µL first PCR.
[b] Based on recommended starting input of 30,000 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 input T cells.
N/A = not applicable

NOTE: DNA from formalin-fixed, paraffin-embedded (FFPE) samples and cDNA are not currently supported for use with the immunoSEQ hsTCRB Kit. For more information, please contact Technical Services.

RECOMMENDATIONS FOR SAMPLE PREPARATION

Isolating DNA from different sample types

Sorted T cells, or sorted cells

- Sorting fixed cells into HEPES buffer (PBS with 2%FBS and 0.025M HEPES) 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

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 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:
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