Human T-cell Assay Sample Preparation Guidelines

Work with archived, fresh or frozen lymphocyte-containing samples

PROFILING RESOLUTIONS: SURVEY[1] VS. DEEP[1]

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 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 cells

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

TARGET QUANTITIES FOR EXTRACTED gDNA

Sample Type (Target concentration or mass)	TCR Beta Assay		TCR Gamma Assay	
	Survey (in 100 µL TE)	Deep (in 100 µL TE)	Survey (in 50 µL TE)	Deep (in 120 µL TE)
Sorted cells	10 ng/µL 1 µg	30 ng/µL 3 µg	20 ng/µL 1 µg	25 ng/µL 3 µg
Peripheral blood mononuclear cells	17 ng/µL 1.7 µg	50 ng/µL 5 µg	34 ng/µL 1.7 µg	42 ng/µL 5 µg
Whole blood	50 ng/µL 5 µg	150 ng/µL 15 µg	100 ng/µL 5 µg	125 ng/µL 15 µg
Bone marrow aspirate	100 ng/µL 10 µg	300 ng/µL 30 µg	200 ng/µL 10 µg	250 ng/µL 30 µg
Bone marrow mononuclear cells	30 ng/µL 3 µg	90 ng/µL 9 µg	60 ng/µL 3 µg	75 ng/µL 9 µg
Lymphoid tissue	17 ng/µL 1.7 µg	50 ng/µL 5 µg	34 ng/µL 1.7 µg	42 ng/µL 5 µg
Non-lymphoid tissue	30 ng/µL 3 µg	90 ng/µL 9 µg	60 ng/µL 3 µg	75 ng/µL 9 µg
Formalin-fixed, paraffin-embedded	25 microns in 100 µL (max scroll thickness of 10 microns each)	50 microns in 100 µL (max scroll thickness of 10 microns each)	25 microns in 50 µL (max scroll thickness of 10 microns each)	50 microns in 120 µL (max scroll thickness of 10 microns each)

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 cells is 1,000. For more information see our Service Sample FAQs (immunseq.com/knowledge-center/immunoseq-assays)
- immunoSEQ Assays are compatible with less gDNA than outlined in the chart above; however, submitting gDNA at a concentration less than 10 ng/µL limits our ability to troubleshoot issues.

Assays available:

- T-cell receptor beta (TCRB)
- T-cell receptor gamma (TCRG)

Shipping:

When you place an order, we will send you a return shipping box containing lab-ware and sample shipping 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

TCRG

Both alpha/beta and gamma/delta T cells carry a rearranged gamma locus.[2] As a result, sequencing directly from tissue or unsorted cells will result in the generation of gamma sequences from both T-cell populations. In order to sequence only the gamma/delta T cells, a cell sort must be performed prior to submitting your samples.

NOTE:

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

1. Survey and Deep are names to represent the breadth of the immune repertoire assayed (e.g. number of T cells); the names do not represent the quality of data or results generated
2. Sherwood, et al. *Science Translational Medicine*, 2011

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 gDNA, 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
- While sodium heparin and sodium citrate have been 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

Formalin-Fixed, Paraffin-Embedded (FFPE)

- Due to cross-linking of nucleic acids and increased gDNA shearing, FFPE samples may yield low quantities of viable gDNA for immunosequencing
- Many factors can impact the yield and quality of FFPE derived gDNA. For best results we recommend using:
  - Buffered fixatives
  - Low fixative concentration
  - Short fixation time
  - Low processing temperature
  - Limiting storage time

Extraction Kits

Any validated gDNA extraction method may be used to prepare sample gDNA for the immunoSEQ Assay. We do not exclusively recommend or provide technical support for any of the gDNA 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)
- QIAGEN QIAmp® DNA Blood Maxi or Micro Kit

For FFPE samples:

- QIAGEN Deparaffinization Solution
- QIAGEN QIAmp DNA FFPE Tissue 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

- 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

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 gDNA extraction

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

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

Information in this document pertains to version 4 of the TCRB assay and version 3 of the TCRG assay.

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