Human TCRB assay sample preparation guidelines

A wide range of sample sizes

The sample type, the number of expected T cells and the experimental question are important factors to consider when deciding how much material to submit.

Adaptive Immunosequencing can be used to sequence samples from 1,000 to 600,000 T cells, providing excellent sensitivity and insight for both clonal and diverse samples. Additionally, because the assay uses genomic DNA (gDNA), many sample types can be used, including FFPE. Elute to the requested volume independent of concentration.

Extracted gDNA ranges

Absolute minimum to recommended maximum

SAMPLE TYPE	gDNA AMOUNTS (IN 100 µL TE) TARGET MASS OR CONCENTRATION
Sorted cells	0.1 ng/µL - 72 ng/µL (0.01 µg - 7.2 µg)
Peripheral blood mononuclear cells (PBMCs)[a]	0.34 ng/µL - 206 ng/µL (0.034 µg - 20.6 µg)
Whole blood or buffy coat[a]	0.5 ng/µL - 360 ng/µL (0.05 µg - 36 µg)
Bone marrow aspirate	1.0 ng/µL - 300 ng/µL (0.1 µg - 30 µg)
Bone marrow mononuclear cells (BMMCs)	0.3 ng/µL - 220 ng/µL (0.03 µg - 22 µg)
Lymphoid tissue	0.17 ng/µL - 120 ng/µL (0.017 µg - 12 µg)
Non-lymphoid tissue	5.5 ng/µL - 90 ng/µL (0.55 µg - 9.0 µg)
Formalin-fixed, paraffin-embedded (FFPE)	25 µm to 50 µm total for extraction[b]

a. Compatible with the Immunosequencing classifiers; due to minimum sample sizes for classifiers, submit as much material as possible.
b. Maximum scroll thickness of 10 microns each.

Considerations for extracting gDNA

Immunosequencing assays are compatible with a wide range of gDNA concentrations; however, submitting gDNA at a concentration less than 10 ng/µL limits our ability to troubleshoot issues.

Work with archived, fresh or frozen lymphocyte containing samples

FLEXIBLE SAMPLE TYPES

- Formalin-fixed, paraffin-embedded (FFPE), fresh/frozen and optimal cutting temperature (OCT) tissue
- Sorted or stimulated T cells
- Peripheral blood mononuclear cells (PBMCs)
- Whole blood
- Buffy coat
- Bone marrow aspirate
- Bone marrow mononuclear cells (BMMCs)
- Lymphoid and non-lymphoid tissue
- gDNA from T-cell-containing samples
- Complementary DNA (cDNA) synthesized from a minimum of 150 ng of RNA is compatible with the assay

TUBES ACCEPTED

Acceptable tubes include 1.7-2 mL microtubes or cryogenic tubes for cell pellets, tissues, FFPE scrolls or slide scrapings. Blood is ideally submitted in a purple-top (EDTA) vacutainer up to 10 mL. Conical vials, as well as 1.7-2 mL microtubes or cryogenic tubes are acceptable for blood, but not preferred. Except for vacutainers, Adaptive cannot accept tubes larger than 2 mL or smaller than 1.7 mL. Specimens sent in multiple tubes cannot be combined for sample processing.

SHIPPING

We will send you a return shipping box containing labware and sample shipping instructions. Please use the materials provided to send samples to Adaptive Biotechnologies.

FOR QUESTIONS, CONTACT

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Recommendations for extracting gDNA

ISOLATING DNA FROM DIFFERENT SAMPLE TYPES

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), we recommend a concentration of 0.5%–2% paraformaldehyde (PFA); higher concentrations of PFA can fragment gDNA, which will result in reduced PCR amplification efficiency.

Tissue

- We recommend a tissue homogenizer with a homogenization buffer for disruption of fresh or frozen tissue samples.
- DNA from OCT tissue is acceptable. We accept OCT tissue as 5- to 10-micron sections. Remove as much of the OCT as possible by washing it before performing DNA extractions or aliquoting tissue samples for shipment.

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, use:
  - Buffered fixatives
  - Low fixative concentration
  - Short fixation time
  - Low processing temperature
  - Limited storage time

Blood, peripheral blood mononuclear cells (PBMCs) or bone marrow

- EDTA is the recommended anticoagulant for whole blood or bone marrow collection; however, excessive amounts of EDTA can inhibit PCR.
- Sodium heparin and sodium citrate are compatible with the Immunosequencing assay; however, excessive amounts can inhibit PCR.
- Approximately 50% of cells frozen in dimethyl sulfoxide (DMSO) will lyse during the thawing process; for best results, extract gDNA from the entire thawed sample without centrifuging.

EXTRACTION KITS

Any validated gDNA extraction method may be used to prepare samples for Immunosequencing. We do not exclusively recommend or provide technical support for any of the gDNA extraction products named. Contact the kit manufacturer with questions or for technical support.

Example extraction kits

- QIAGEN DNeasy Blood & Tissue Kit (Mini Spin Columns)
- QIAGEN QIAamp DNA Blood Maxi or Micro Kit

For FFPE samples

- QIAGEN Deparaffinization Solution
- QIAGEN QIAamp DNA FFPE Tissue Kit

QUALITY OF GDNA

Once DNA is isolated, we highly recommend quantification using a spectrophotometer or comparable method. For optimal results, the absorbance ratios of DNA samples should be:

- 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. Template clonality and quantification do not apply for cDNA; rather, template frequency reflects relative receptor expression levels.

POTENTIAL PCR INHIBITORS

Sample source(s) containing any of the following may inhibit PCR steps used in the 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, contact: **Academic research customers** customercare@adaptivebiotech.com | **Pharma and biotech customers** bdclientmanagers@adaptivebiotech.com

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