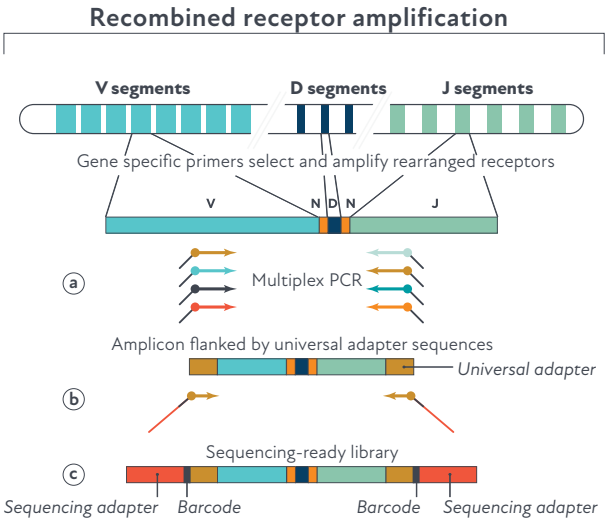
immunoSEQ By Adaptive

Mouse TCRB assay: Sample preparation guidelines

The power of immunosequencing

Accuracy of immunosequencing depends on controlling for PCR amplification bias. The immunoSEQ assay use a combination of highly optimized multiplex PCR primers, a set of built-in controls consisting of synthetic immune receptor sequence analogs, and advanced bioinformatics. This robust solution allows for exceptionally accurate quantification of thousands to millions of T cells.



V, D and J segments are recombined to form the CDR3 of the TCRB locus.
a. Multiplex PCR to capture the highly variable CDR3
b. PCR to add barcodes and adaptor sequences for high-throughput sequencing
c. Sequencing-ready libraries

Sample guidelines

Sample type (target mass or concentration)	Profiling resolution	
	Survey (in 50 µL TE)	Deep (in 100 µL TE)
Sorted cells	3,000-150,000 T cells 10-500 ng 0.25-10 ng/µL	1.5 µg DNA 15.0 ng/µL
Whole blood	50-100 µL blood 12-600 ng 0.3-12 ng/µL	200.0 µL blood 1.8 µg DNA 18.0 ng/µL
Lymphoid tissue	up to 10 mg tissue 20-1,000 ng 0.5-20 ng/µL	3.0 µg DNA 30.0 ng/µL
Non-lymphoid tissue	50-100 µL blood 12-600 ng 0.3-12 ng/µL	—

FLEXIBLE SAMPLE TYPES

- Sorted T cells
- Whole blood
- Tissue (including fresh frozen)
- gDNA and cDNA

COVERAGE

For complementary DNA (cDNA), we do not target a minimum coverage. Moreover, clonality and quantification of templates do not apply for cDNA; rather, template frequency reflects the relative expression levels of receptors.

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.

FOR QUESTIONS, CONTACT

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Notes: 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, we request an absolute minimum of 400 ng of genomic DNA (gDNA).

Considerations for extracting gDNA

- It's critical to know the extraction efficiency of your extraction method and to accurately estimate the input requirement in order to reach the amounts of genomic DNA (gDNA) and concentrations outlined in the chart above.
- The minimum number of input T cells is 1,000.
- The assay is 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.

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), a concentration of 0.5%–2% 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

- K2EDTA tubes (lavendar-top tubes) are recommended, because they contain 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 assay, excessive amounts of sodium heparin 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 sample gDNA for the assay. 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

QUALITY OF GDNA

Once DNA is isolated, quantification using a spectrophotometer or comparable method is 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 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:

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PM-US-iSEQ-0021 (03/2023)