Human TCRB Kit Overview

The immunoSEQ Assay is the gold-standard for accurate, quantitative TCRB sequencing. Leverage the same multiplex PCR assay, high-throughput sequencing and sophisticated computational tools in your own lab:

- **Compatible with FFPE tissue** derived DNA
- **Control** when and where your samples are sequenced
- **Customizable setup** offering flexibility in experimental design
- **Simple workflow** with less than 4 hours hands on time
- **Primer design reduces background sequences** for increased precision when measuring T-cell fraction in tissue samples

**THE POWER OF IMMUNOSEQUENCING**

Accuracy of immunosequencing depends on controlling for PCR amplification bias. Our hsTCRB Assay uses a combination of highly optimized multiplex PCR primers (Figure 1), a set of synthetic immune molecules as built-in controls, and advanced bioinformatics to control for PCR amplification bias. Additionally, reference gene primers allow for quantifying total nucleated cells and calculating T-cell fraction in each sample. This robust solution delivers accurate quantification of thousands to millions of T cells.

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**Acceptable Sample Types:**
- FFPE or fresh/frozen tissue samples
- Bone marrow
- Bone marrow mononuclear cells (BMMCs)
- Whole blood and PBMCs
- Sorted or stimulated T cells
- gDNA from T-cell containing samples

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**RECOMBINED RECEPTOR AMPLIFICATION**

Gene specific primers select and amplify rearranged receptors

**REFERENCE GENE AMPLIFICATION**

Gene specific primers select and amplify reference genes

**Figure 1.** V, D, and J segments are recombined to form the CDR3 of the human TCRB locus.

A) Multiplex PCR to capture the highly variable CDR3 and reference genes.
B) PCR to add barcodes and adaptor sequences for high-throughput sequencing.
C) Sequencing-ready libraries.

For Research Use Only. Not for use in diagnostic procedures.
CONVENIENT LIBRARY PREP IN YOUR LAB   Hands-on time: < 4 hours  |  Sample to sequencing: < 8 hours

Materials Required (provided):
• Barcode plate (12 μL per well)
• Primer mix (1 tube, 250 μL)
• Positive control (250 μL)
• PCR cleanup beads (1 bottle, 3 mL)
• 2 QIAGEN® Multiplex PCR Plus Kit (100) (QIAGEN catalog number: 206152) [billed separately]
• Full access to the immunoSEQ® Analyzer is included

Materials Required (not provided):
• Illumina® MiSeq® system or NextSeq® 500 system
• Illumina MiSeq Reagent Kit(s) v3 (150 cycle) or Illumina NextSeq 500 Mid Output Kit(s) v2 (150-cycle)
• Illumina PhiX Control v3
• Instrument for measuring DNA concentration
• Cold block compatible with PCR plates
• Plate centrifuge capable of 4000 x g
• Magnet stand compatible with your 1.5 or 2.0 mL tubes
• Thermal cycler with heated lid
  Recommended: Bio-Rad® S1000™ Thermal Cycler or C1000 Touch™ Thermal Cycler with 95-Deep Well Reaction Module

Materials Recommended (not provided):
• Nuclease-free reagent reservoirs
• 8- or 12-channel pipettes (20 and 200 μL) with aerosol-resistant tips
• High-speed microplate shaker
  Recommended: Eppendorf® MixMate® (P/N 5353 000.014) with a 1.5 mm mixing orbit
• PCR plate labels
• Instrument(s) for visualizing, quantitating, and sizing DNA

Storage:
• 2–8°C

Box Dimensions:
• 2.5” X 6.5” X 7”

FLEXIBLE SAMPLE THROUGHPUT

Input Requirements:

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>ng gDNA—Survey</th>
<th>ng gDNA—Deep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorted T cells</td>
<td>400–500</td>
<td>1,200–1,500</td>
</tr>
<tr>
<td>PBMCs</td>
<td>1,100–1,600</td>
<td>3,380–4,700</td>
</tr>
<tr>
<td>Whole blood</td>
<td>1,590–2,600</td>
<td>4,680–7,800</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>2,700–9,000</td>
<td>N/A</td>
</tr>
<tr>
<td>BMMCs</td>
<td>1,590–2,600</td>
<td>4,680–7,800</td>
</tr>
<tr>
<td>Lymphoid Tissue</td>
<td>650–1,000</td>
<td>1,900–3,000</td>
</tr>
<tr>
<td>Non-Lymphoid Tissue</td>
<td>2,700–12,000c</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Number of Samples Per Sequencing Run:

<table>
<thead>
<tr>
<th>System</th>
<th>Number of reads</th>
<th>Number of samples d for Survey (2 replicates)</th>
<th>Number of samples d for Deep (4 replicates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiSeq Kit v3</td>
<td>up to 25 million</td>
<td>up to 25d</td>
<td>up to 9d</td>
</tr>
<tr>
<td>NextSeq 500 Mid Output Kit v2</td>
<td>up to 130 million</td>
<td>up to 47</td>
<td>up to 23</td>
</tr>
</tbody>
</table>

Based on recommended 30,000 to 45,000 input T-cell genomes per reaction
Based on 25M total reads generated per MiSeq run, or 130M reads generated per NextSeq run.
Does not achieve 30,000 input T-cell genomes per reaction,  Varies by sample type

ORDERING INFORMATION:
immunoSEQ hsTCRB Kit
Part Number – ISK10050
Contact your Immunosequencing Account Executive or (855) 466-8667 | customercare@adaptivebiotech.com
www.adaptivebiotech.com