Miltenyi Biotec

TCR α/β RNA Profiling Kit
human

Order no. 130-139-385

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1. Description

This product is for research use only.

Components	**TCR α/β RNA Profiling Kit Box 1:**
	52 µL TCR-A/B cDNA Synthesis Primers (blue lid label)
	52 µL TCR-A PCR1 Primers (pink lid label)
	52 µL TCR-B PCR1 Primers (yellow lid label)
	26 µL TCR-A PCR2 Primers (red lid label)
	26 µL TCR-B PCR2 Primers (orange lid label)
	24 rxn RT Master Mix (no lid label)
	3× 600 µL PCR Master Mix (green lid label)
	Immunoprofiling Reagents Box 2:
	2× 1.6 mL Water
	2× 1.6 mL Elution Buffer
	540 µL Lysis Solution
Capacity	For 24 samples.
Storage	Store components of the TCR α/β RNA Profiling Kit Box 1 at –20 °C immediately upon arrival. Store components of the Immunoprofiling Reagents Box 2 at room temperature (+15 to +25 °C). The expiration date is indicated on the vial label.

1.1 Background information

The TCR α/β RNA Profiling Kit, human has been designed for the generation of TCRα and TCRβ cDNA libraries using the combination of highly sensitive multiplex PCR and unique molecular identifiers (UMIs). UMIs are introduced along with first strand cDNA synthesis and allow for advanced PCR and sequencing error correction, elimination of PCR biases, exact quantification of template cDNA molecules, and accurate normalization of samples for comparison of repertoire diversity metrics.

The TCR α/β RNA Profiling Kit, human supports an RNA input equivalent to 100 to 500,000 sorted or purified T cells, derived from peripheral blood leukocytes, or from T cell–containing tissues, and produces indexed ready-to-sequence libraries for Illumina®-based sequencing. For optimal, high quality RNA extraction and cell isolation, it is recommend to use the gentleMACS Dissociators. For further cell separation and enrichment of specific cell types from cell suspensions, it is recommended to explore MACS Cell Separation technology. During cDNA library construction, reverse transcription is performed jointly for TCRα and TCRβ chains.

The first PCR (multiplex amplification) is performed separately for TCRα and TCRβ chain repertoires by splitting the cDNA input into two aliquots. Sample barcodes are introduced during the second (indexing) PCR. TCR profiling requires 150×150 bp paired-end Illumina sequencing, ensuring sufficient reads per UMI sequencing coverage.

For more information and a step-by-step experimental protocol, please refer to the application protocol at **www.miltenyibiotec.com/applicationprotocols**.

1.2 Applications

TCR α/β repertoire analysis to investigate:

- repertoire diversity and development,
- identification of clonotypes and biomarkers,
- immune responses to infections.

1.3 Reagent and instrument requirements

- Thermal cycler
- Bioanalyzer® and/or TapeStation® System (Agilent®)
- Qubit® fluorimeter (Thermo Fisher®)
- Low-speed benchtop mini-centrifuge
- Vortex mixer
- Magnetic separation rack for 0.2 mL PCR tubes
- AMPure® XP Beads (Beckman Coulter®) or SPRIselect® reagent (Beckman Coulter®)
- Illumina® DNA/RNA UD Indexes, Tagmentation (Set A – D)
- Freshly prepared 80% ethanol (1200 µL per sample)
- (For cDNA synthesis directly from cells) Phosphate-buffered saline (PBS)
- (Optional) Peripheral blood mononuclear cell (PBMC) RNA or any other suitable control RNA in case a positive control is desired for the experiment.

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2. Protocol

The detailed protocol for the kit is available at **www.miltenyibiotec.com/applicationprotocols**.

2.1 General recommendations to prevent contamination

General recommendations to lower the risk of RNA degradation and contamination should be implemented, such as using lab coats, gloves, tips with aerosol filters, certified RNAse/DNAse-free reagents, and decontamination of the workspace before starting to work with RNA.

Performing non-template control reactions is strongly recommended for each experiment. To prevent cross-sample contamination, library preparation should be performed in two physically separated workspaces (PCR boxes): one for pre-PCR and one for post-PCR, located in different rooms. The user should avoid transferring anything (reagents, pipets, racks, etc.) from the post–PCR to the pre–PCR workspace. In the case of possible contamination, use decontaminating procedures with special reagents.

Perform cell isolation, RNA purification, cDNA synthesis, and first and second PCR Master Mix preparations in the pre–PCR workspace. After the 1st PCR amplification, transfer the tubes to the post–PCR workspace and perform all other steps (except PCR Master Mix preparation) in the post–PCR workspace.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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