Immunoverse™-HS TCR Workflow Overview

Follow the instructions and incubations for each step. All mixing steps should be performed on ice. Pipette up and down 8 times or vortex to mix after re-suspending each lyosphere and spin down prior to incubations and transfers. For incubations, use a lid heated ≥100°C except where specified otherwise.

TCR Specific RT Priming

Add optimal amount (see Appendix) of RNA input to TCR Specific RT Priming reagent for a total volume of 20 µL.

Incubate as indicated

Step	Incubation Temperature	Incubation Time
1	65°C	5 min
2	4°C	Hold

First Strand cDNA Synthesis

Transfer 20 µL TCR Specific RT Priming mixture to First Strand cDNA Synthesis reagent.

Incubate as indicated

Step	Incubation Temperature	Incubation Time
1	50°C	30 min
2	80°C	20 min
3	4°C	Hold

Second Strand cDNA Synthesis

Add 20 µL ultrapure water to 20 µL First Strand cDNA Synthesis mixture and then transfer to Second Strand cDNA Synthesis reagent.

Incubate as indicated

STOP **Optional stopping point after this step. Store at -10°C to -30°C.**

Step	Incubation Temperature	Incubation Time
1	16°C	60 min
2	75°C	20 min
3	4°C	Hold

End Repair

Transfer 40 µL Second Strand cDNA Synthesis mixture to End Repair reagent.

Incubate as indicated with <u>unheated lid</u>

Step	Incubation Temperature	Incubation Time
1	25°C	30 min
2	4°C	Hold

AMPure™ XP clean-up (100 µL). Elute in 20 µL

Ligation Step 1

Transfer 20 µL End Repair mixture to Ligation Step 1 reagent.

Incubate as indicated

After incubation add 20 µL of 10 mM Tris-HCl, pH 8.0 and mix.

Step	Incubation Temperature	Incubation Time
1	37°C	15 min
2	4°C	Hold

MBC Adapters

Transfer 40 µL Ligation Step 1 mixture to the MBC adapters.

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Ligation Step 2

Transfer the entire volume of the MBC adapters to Ligation Step 2 reagent.

Incubate as indicated with <u>unheated lid</u>

STOP **Optional stopping point before purification. Store at -10°C to -30°C.**

Step	Incubation Temperature	Incubation Time
1	25°C	15 min
2	4°C	Hold

Ligation clean-up beads (50 µL). Elute in 36 µL 5mM NaOH, 75°C 10 min

First PCR

Add 4 µL GSP1 primers to the First PCR HS reagent. Then add 36 µL of purified Adapter Ligation mixture and mix.

Incubate as indicated

STOP **Optional stopping point after this step. Store at -10°C to -30°C.**

Incubation Temperature	Incubation Time	# of cycles
95°C	3 min	1
95°C	30 sec	24
65°C	3 min (ramp rate 100%)	
72°C	3 min	1
4°C	Hold	1

AMPure™ XP clean-up (48 µL). Elute in 44 µL

Second PCR

Transfer 40 µL of purified First PCR mixture and mix.

Incubate as indicated

STOP **Optional stopping point after this step. Store at -10°C to -30°C.**

Incubation Temperature	Incubation Time	# of cycles
95°C	3 min	1
95°C	30 sec	8
65°C	3 min (ramp rate 100%)	
72°C	3 min	1
4°C	Hold	1

AMPure™ XP clean-up (48 µL). Elute in 20 µL

Proceed with Protocol: Quantify, Normalize, and Sequence Protocol for Illumina®

Appendix: RNA Input Recommendations and Sequencing Read Depth

Panel	Application	RNA Input Recommendations*	Sequencing Read Depth
TCR B/G	Clonality or Dominant Clones	25-400 ng	250,000
TCR A/D	Clonality or Dominant Clones	25-400 ng	250,000
TCR All Chains	Clonality or Dominant Clones	25-400 ng	500,000
TCR B/G	Thorough Characterization of Repertoire or Rare Clonotype Identification	400-2000 ng	1.5 M
TCR A/D	Thorough Characterization of Repertoire or Rare Clonotype Identification	400-2000 ng	1.5 M
TCR All Chains	Thorough Characterization of Repertoire or Rare Clonotype Identification	400-2000 ng	3 M