Technical Data Sheet

T-Cell Protein Panel

Product Information

Material Number:	572178
Size:	2 Tests
Reactivity:	Tested in Development: Human
Storage Buffer:	Lyophilized powder containing BSA and ≤ 0.25% sodium azide
Component:	51-9024945
Description:	T-Cell Protein Panel
Size:	1 Test (2 each)

Description

The BD® OMICS-One T-Cell Protein Panel consists of 30 different specificities against major T-cell markers in a single tube. Designed and optimized to work on the BD Rhapsody™ System, the T-Cell Protein Panel is tested to work seamlessly alongside the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Assay, Targeted mRNA Assay, BD® Single-Cell Multiplexing Kit (SMK), BD® Intracellular CITE-seq (IC-AbSeq) Assay, and BD Rhapsody™ TCR/BCR Next Multiomic Assay for human. The individual antibodies were each conjugated to an oligonucleotide that contains a specific antibody barcode sequence flanked by a polyA tail on the 3' end and a common PCR handle (PCR primer binding site) on the 5' end. All AbSeq barcode sequences were generated in silico with minimal sequence similarity to the human genomes, have low predicted secondary structure, and have high Hamming distance within the BD antibody-oligo portfolio, to allow for sequencing error correction and unique mapping. The polyA tail of the oligonucleotide allows the barcode sequence to be captured by the BD Rhapsody™ Enhanced Cell Capture Beads. The 5' PCR handle allows for efficient sequencing library generation for various sequencing platforms. Each individual antibody exists at an optimal concentration within the 30-plex to enable superior target and population resolution.

The T-Cell Protein Panel is designed with SMART technology. SMART technology helps lower sequencing cost while increasing data resolution by attenuating antibodies that target high-expressing primary markers and allowing re-allocation of sequencing reads to markers expressed at lower levels. With SMART technology, now markers low in expression can be quantified without having to do deeper sequencing and incurring high sequencing cost. The two specificities attenuated in the T-Cell Protein Panel are CD4 and CD44.

Preparation and Storage

Store at 2−8°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application:	Single Cell 3' Sequencing (Qualified)
Barcode Sequence:	Specific for each individual AbSeq antibody (see panel table on page 3)
Sequence ID:	Specific for each individual AbSeq antibody (see panel table on page 3)

Recommended Assay Procedure

This reagent is provided lyophilized in a pre-titrated format.

1. Remove the BD® OMICS-One T-Cell Protein Panel tube from the foil bag and bring up to room temperature for 5 minutes.
2. Make sure the pellet is located at the bottom of the tube. If not, briefly centrifuge to collect the contents at the tube bottom.
3. Add 35 µL of nuclease-free water to the bottom of the tube and allow antibodies to reconstitute for 5 minutes at room temperature.
4. Transfer the reconstituted antibodies on ice until the cells are ready for staining.
   Note: Reconstitute antibody immediately before cell staining. Prolonged incubation of reconstituted antibody may increase the non-specific background.

BD Biosciences
bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	866.979.9408	32.2.400.98.95	0120.8555.90	65.6861.0633	55.11.5185.9995

For country contact information, visit **bdbiosciences.com/contact**.

5. For BD® AbSeq Ab-Oligo drop-in of 60 plex or lower, prepare the BD® AbSeq labeling MasterMix in 1.5-mL LoBind tube on ice.
Note: For drop-in with more than 60 plex, reach out to technical support for calculation.

   For sequential labeling with Sample Tags or no Sample Tags, prepare BD® AbSeq labeling MasterMix for drop-ins as specified in the following table.

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
Per BD® AbSeq Ab-Oligo	2.0	2.6	5.2
Total of AbSeq Ab-Oligo (N = Number of drop-in antibodies) N=0 if no drop-in antibodies	2.0 × N	2.6 × N	5.2 × N
BD Pharmingen™ Stain Buffer (FBS) (catalog number 554656)	140 – (2.0 × N)	182 – (2.6 × N)	364 – (5.2 × N)
Total	**140**	**182**	**364**

   For co-labeling with Sample Tags, prepare BD® AbSeq labeling MasterMix for drop-ins as specified in the following table.

Component	1 sample (µL)	1 sample + 30% overage (µL)	2 samples + 30% overage (µL)
Per BD® AbSeq Ab-Oligo	2.0	2.6	5.2
Total of AbSeq Ab-Oligo (N = Number of drop-in antibodies) N=0 if no drop-in antibodies	2.0 × N	2.6 × N	5.2 × N
BD Pharmingen™ Stain Buffer (FBS) (catalog number 554656)	120 – (2.0 × N)	156 – (2.6 × N)	312 – (5.2 × N)
Total	**120**	**156**	**312**

6. Pipet-mix the BD® AbSeq labeling MasterMix for drop-ins. Briefly centrifuge to collect the contents at the bottom, and place back on ice.
7. For sequential labeling with Sample Tags or no Sample Tags, for each sample, add 140 µL BD® AbSeq labeling MasterMix of drop-ins to the tube containing 35 µL reconstituted T-Cell Protein Panel solution to make a total volume of 175 µL.
   For co-labeling with Sample Tags, for each sample, add 120 µL BD® AbSeq labeling MasterMix of drop-ins and 20 µL Sample Tag to the tube containing 35 µL reconstituted T-Cell Protein Panel solution to make a total volume of 175 µL.
8. Pipet-mix the mixture, briefly centrifuge to collect the contents at the tube bottom, and place back on ice.
9. Centrifuge cells at 400 × *g* for 5 minutes. If Fc Block is used, proceed to step 10. If Fc Block is not used. skip to step 11.
10. (Optional) For samples containing myeloid and B lymphocytes, BD Biosciences recommends blocking nonspecific Fc Receptor–mediated false-positive signals with Human BD Fc Block (catalog number 564220).
    a. To perform blocking, pipet the Fc Block MasterMix into a new 1.5-mL LoBind tube on ice:

Component	1 sample (µL)*	1 sample + 20% overage (µL)
BD Pharmingen™ Stain Buffer (FBS) (catalog number 554656)	20.0	24.0
BD Pharmingen™ Human BD Fc Block (catalog number 564220)	5.0	6.0
Total	**25.0**	**30.0**

    * Sufficient for up to 1,000,000 cells. To block more cells, adjust the volume.
    b. Pipet-mix the Fc Block MasterMix and briefly centrifuge. Place on ice.
    c. Remove the supernatant from the cells without disturbing the pellet.
    d. Resuspend the cells in 25 µL of Fc Block MasterMix.
    e. Incubate the cells at room temperature (15°C to 25°C) for 10 minutes.
    f. Add 175 µL of BD® AbSeq labeling MasterMix from Step 8 into the cell suspension. Pipet-mix and proceed to Step 12.
11. Remove the supernatant from the cells without disturbing the pellet. Add 25 µL Stain Buffer (FBS) to the 175 µL of BD® AbSeq labeling MasterMix from Step 8 to make a total volume of 200 µL. Resuspend the cell pellet in 200 µL total volume. Pipet-mix.
12. Transfer the cells with BD® AbSeq labeling MasterMix into a new 5-mL polystyrene Falcon tube.
13. Stain the cells on ice for 30 minutes.
14. Add 3-4 mL Stain Buffer (FBS) to labelled cells and pipet-mix.
15. Centrifuge at 400 × *g* for 5 minutes.
16. Uncap the tube and invert to decant supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
17. Repeat steps 14–16 twice more for a total of 3 washes.
18. Resuspend the final washed cell pellet in 620 µL cold Sample Buffer from the BD Rhapsody™ Enhanced Cartridge Reagent V3 (catalog number 667052) and proceed to single cell capture with on-cartridge washing described in sub-steps a–c. Refer to the *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID 23-24252) or *BD Rhapsody™ HT Xpress System Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID 23-24253) for additional details.
    Note: Perform on-cartridge washing after cell settling (8-minute incubation) as described in the following sub-steps.
    a. At the protocol section of "Loading cells in BD Rhapsody™ 8-Lane Cartridge", after cell load, incubate the cartridge in dark at room temperature for 8 minutes.

b. Place the cartridge on the BD Rhapsody™HT Xpress and perform the On-Cartridge Wash steps as follows:

Material to load	Volume (uL) 1 lane	Pipette Mode
Air	380	Prime/Wash
Cold Sample Buffer	380	Prime/Wash
Air	380	Prime/Wash
Cold Sample Buffer	380	Prime/Wash

c. (Optional) Perform the scanner step: Cell Load Scan, if using *BD Rhapsody™ HT Single-Cell Analysis System Single-Cell Capture and cDNA Synthesis Protocol* (Doc ID 23-24252). No need for 8-minute delay before scanning.

Warning: All biological specimens and materials are considered biohazardous. Handle as if capable of transmitting infection and dispose using proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

List of all 30 Human AbSeq specificities included in the BD® OMICS-One T-Cell panel:

Specificity	Clone	Oligo ID	BD® AbSeq Barcode Sequence
CD103	Ber-ACT8	AHS0001	AAATAGTATCGAGCGTAGTTAAGTTGCGTAGCCGTT
CD137	4B4-1	AHS0003	TGACAAGCAACGAGCGATACGAAAGGCGAAATTAGT
CD45RA	HI100	AHS0009	AAGCGATTGCGAAGGGTTAGTCAGTACGTTATGTTG
CD69	FN50	AHS0010	CAATAACGGGTCATAGTAAGTCGCGAGTAAGAGGGC
CD278	DX29	AHS0012	ATAGTCCGCCGTAATCGTTGTGTCGCTGAAAGGGTT
CD134 (OX40)	ACT35	AHS0013	GGTGTTGGTAAGACGGACGGAGTAGATATTCGAGGT
CD279 (PD-1)	EH12.1	AHS0014	ATGGTAGTATCACGACGTAGTAGGGTAATTGGCAGT
CD366 (TIM-3)	7D3	AHS0016	TAGGTAGTAGTCCCGTATATCCGATCCGTGTTGTTT
CD223 (Lag3)	T47-530	AHS0018	CGGCATGAATTAGGCGAGACTTAGTATACGAGCTGG
CD95 (Fas)	DX2	AHS0023	GGCCCGTTAGAGTTGGTATCCGTATGAAGGTTAGCT
CD25	2A3	AHS0026	AGTTGTATGGGTTAGCCGAGAGTAGTGCGTATGATT
CD127	HIL-7R-M21	AHS0028	AGTTATTAGGCTCGTAGGTATGTTTAGGTTATCGCG
CD183	1C6/CXCR3	AHS0031	AAAGTGTTGGCGTTATGTGTTCGTTAGCGGTGTGGG
CD4	SK3	AHS0032	TCGGTGTTATGAGTAGGTCGTCGTGCGGTTTGATGT
CD196 (CCR6)	11A9	AHS0034	ACGTGTTATGGTGTTGTTCGAATTGTGGTAGTCAGT
CD45RO	UCHL1	AHS0036	TGAGAGGTTATTGGGCGTATGACTTCGGTGATTGTG
CD194 (CCR4)	1G1	AHS0038	AATATTAGTGGGTCCTCGCGTTGGCCGGTTGTTAGT
CD62L	DREG-56	AHS0049	ATGGTAAATATGGGCGAATGCGGGTTGTGCTAAAGT
CD272	J168-540	AHS0052	GTAGGTTGATAGTCGGCGATAGTGCGGTTGAAAGCT
CD154	TRAP1	AHS0077	TAAGAGGTAAGTGCATTCGGGTATAGGCGTGATTTG
CD357 (GITR)	V27-580	AHS0104	TCTGTGTGTCGGGTTGAATCGTAGTGAGTTAGCGTG
CD28	L293	AHS0138	TTGTTGAGGATACGATGAAGCGGTTTAAGGGTGTGG
TCRgd	11F2	AHS0142	CTCGTGGGTTAGGCTTGATCGTAGTTATGTATGGTT
CD44	L178	AHS0167	GTGATTGATTAGGACAGTTCGTTGCTTAGTAGTGGG
TCR Vα24-Jα18	6B11	AHS0175	TTCTGGTTCGGTTGAGCTACTAATTTCGTTGGATGG
CD161 (KLRB1)	HP-3G10	AHS0205	TTTAGGACGATTAGTTGTGCGGCATAGGAGGTGTTC
CD8	SK1	AHS0228	AGGACATAGAGTAGGACGAGGTAGGCTTAAATTGCT
CD3	UCHT1	AHS0231	AGCTAGGTGTTATCGGCAAGTTGTACGGTGAAGTCG
CD197 (CCR7)	2-L1-A	AHS0273	AATGTGTGATCGGCAAAGGGTTCTCGGGTTAATATG
TIGIT	tgMab-2	AHS0411	AGAGGGTTTAGTCAAGGTCGTGCGTATAGTTCAGGT

Suggested Companion Products

Catalog Number	Description	Size	Clone
554656	BD Pharmingen™ Stain Buffer (FBS)	500 mL	(none)
564220	BD Pharmingen™ Human BD Fc Block	0.25 mg	Fc1
633801	BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit	1 each	(none)
633774	BD Rhapsody™ Targeted mRNA and AbSeq Amplification Kit	1 each	(none)
667058	BD Rhapsody™ TCR/BCR Next Amplification Kit	1 each	(none)
633773	BD Rhapsody™ cDNA Kit	1 each	(none)
666262	BD Rhapsody™ 8-lane cartridge	1 each	(none)
667052	BD Rhapsody™ Enhanced Cartridge Reagent V3	1 each	(none)
633781	BD® Human Single-Cell Multiplexing Kit	1 each	(none)
633849	BD® Flex Single-Cell Multiplexing Kit A, Flex Sample Tag 1–6	1 each	(none)
633850	BD® Flex Single-Cell Multiplexing Kit A, Flex Sample Tag 7–12	1 each	(none)
633851	BD® Flex Single-Cell Multiplexing Kit A, Flex Sample Tag 13–18	1 each	(none)
633852	BD® Flex Single-Cell Multiplexing Kit A, Flex Sample Tag 19–24	1 each	(none)
666625	BD Rhapsody™ HT Xpress Package	NA	(none)
633701	BD Rhapsody™ Scanner	NA	(none)
570742	BD Rhapsody™ Intracellular AbSeq Buffer Kit	1 each	(none)
570911	BD® Omics-Guard Sample Preservation Buffer	1 each	(none)
570750	BD® AbSeq Enhancer	1 each	(none)
570751	BD® RNase Inhibitor	1 each	(none)

Product Notices

1. This reagent is provided lyophilized in a pre-titrated format.
2. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.
3. Go to https://www.bdbiosciences.com/en-us/resources/protocols/single-cell-multiomics for additional BD Rhapsody protocols.
4. Go to https://abseq-ref-gen.genomics.bd.com/ to access AbSeq reference files in FASTA format for bioinformatics analyses.
5. **Caution:** Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing. Follow state and local guidelines when disposing of hazardous waste.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
7. For U.S. patents that may apply, go to bd.com/patents.
8. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, go to regdocs.bd.com or contact BD Biosciences technical support at researchapplications@bd.com.