Technical Data Sheet

Immune Profiler Protein Panel

Product Information

Material Number:	**571970**
Size:	2 Tests
Reactivity:	Tested in Development: Human
Storage Buffer:	Lyophilized powder containing BSA and ≤ 0.25% sodium azide.
Component:	**51-9024366**
Description:	Immune Profile Protein Panel
Size:	1 Test (2 ea)

Description

The BD® OMICS-One Immune Profiler (IP) Protein Panel consists of 30 different specificities against major immune markers in a single tube. Designed and optimized to work on the BD Rhapsody™ System, the IP is tested to work seamlessly alongside the BD Rhapsody™ Whole Transcriptome Analysis (WTA) Amplification Kit, Targeted mRNA Kits, BD® Single-Cell Multiplexing Kit (SMK), BD® Intracellular CITE-seq (IC-AbSeq), and BD Rhapsody™ TCR/BCR Next Multiomic Assay for human. Each individual antibody exists at an optimal concentration within the 30-plex to enable superior target and population resolution. 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.

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

Recommended Assay Procedure:

This reagent is provided lyophilized in a pre-titrated format

1. Remove the BD® OMICS-One Immune Profiler 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.
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. Store the reconstituted antibodies on ice until the cells are ready for staining.
   Note: Reconstituted antibodies should be used within 24 hours from reconstitution of the lyophilized format.
5. For drop-in of 60 plex or lower, prepare the BD® AbSeq labeling MasterMix in a 1.5-mL LoBind tube on ice using the procedure in **Table 1** for sequential labeling with or without Sample Tags, or the procedure in **Table 2** for co-labeling with Sample Tags.
   Note: For drop-in with more than 60 plex, reach out to technical support for calculation.
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 IP 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 IP 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 (Cat. No. 564220).
    a. To perform blocking, prepare the Fc Block MasterMix in a new 1.5-mL LoBind tube on ice. **See Table 3.**
    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 from Step 9 in 25 µL of Fc Block MasterMix.

BD Biosciences

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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 7 into the cell suspension. Pipet-mix and proceed to Step 12.

11. Remove the supernatant from the cells prepared in step 9 without disturbing the pellet. Add 25 µL Stain Buffer (FBS) to the 175 µL of BD® AbSeq labeling MasterMix from Step 7 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 mL Stain Buffer (FBS) to labelled cells and pipet-mix.
15. Centrifuge at 400 x g for 5 minutes.
16. Uncap each 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 (Cat. No. 667052) and proceed to single cell capture. See 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**. See Table 4 for AbSeq Specificities, Clone Names, Sequence IDs, and Barcode Sequences.** The Barcode Sequence and Sequence ID are specific to each individual AbSeq antibody.

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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
564220	Human BD Fc Block™	0.25 mg	Fc1
633801	Whole Transcriptome Analysis (WTA) Amplification Kit	1 Each	(none)
633774	Targeted mRNA and AbSeq Amplification Kit	1 Each	(none)
667058	TCR/BCR Next Amplification Kit	1 Each	(none)
633773	cDNA Kit	1 Each	(none)
666262	8-Lane Cartridge	1 Each	(none)
667052	Enhanced Cartridge Reagent Kit V3	1 Each	(none)
633781	Hu Single Cell Sample Multiplexing Kit	1 Each	(none)
633849	Flex Single-Cell Multiplexing Kit A, Flex Sample Tag 1-6	1 Each	(none)
633850	Flex Single-Cell Multiplexing Kit B, Flex Sample Tag 7-12	1 Each	(none)
633851	Flex Single-Cell Multiplexing Kit C, Flex Sample Tag 13-18	1 Each	(none)
633852	Flex Single-Cell Multiplexing Kit D, Flex Sample Tag 19-24	1 Each	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
666625	BD® Rhapsody™ HT Xpress Package		(none)
633701	BD Rhapsody Scanner		(none)
570742	Intracellular AbSeq Buffer Kit	1 Each	(none)
570911	OMICS-Guard Sample Preservation Buffer	50 mL	(none)
570750	AbSeq Enhancer Kit	1 Each	(none)
570751	RNase Inhibitor	1 Each	(none)

Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
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. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
4. For U.S. patents that may apply, see bd.com/patents.
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. Please refer to https://www.bdbiosciences.com/en-us/resources/protocols/single-cell-multiomics for technical protocols.
7. Go to https://abseq-ref-gen.genomics.bd.com/to access AbSeq reference files in FASTA format for bioinformatics analyses.

Table 1: For sequential label 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	2.0 × N	2.6 × N	5.2 × N
BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) (N = Number of drop-in antibodies) N=0 if no drop-in antibodies	140 – (2.0 × N)	182 – (2.6 × N)	364 – (5.2 × N)
Total	**140**	**182**	**364**

Table 2: 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	2.0 × N	2.6 × N	5.2 × N
BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) (N = Number of drop-in antibodies) N=0 if no drop-in antibodies	120 – (2.0 × N)	156 – (2.6 × N)	312 – (5.2 × N)
Total	**120**	**156**	**312**

Table 3: Fc Block MasterMix formulation per sample:

Component	1 sample (µL)*	1 sample + 20% overage (µL)
BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656)	20.0	24.0
BD Pharmingen™ Human BD Fc Block (Cat. No. 564220)	5.0	6.0
Total	**25.0**	**30.0**

* Sufficient for ≤ 1×10^6 cells. To block more cells, adjust the volume.

Table 4: List of all 30 Human AbSeq specificities included in the panel:

Specificity	Clone	Oligo ID	BD® AbSeq Barcode Sequence
CD3	UCHT1	AHS0231	AGCTAGGTGTTATCGGCAAGTTGTACGGTGAAGTCG
CD4	SK3	AHS0032	TCGGTGTTATGAGTAGGTCGTCGTGCGGTTTGATGT
CD8	SK1	AHS0228	AGGACATAGAGTAGGACGAGGTAGGCTTAAATTGCT
CD11c	B-Ly6	AHS0056	ATGCGTTGCGAGAGATATGCGTAGGTTGCTGATTGG
CD14	MPHIP9	AHS0037	TGGCCCGTGGTAGCGCAATGTGAGATCGTAATAAGT
CD16	3G8	AHS0053	TAAATCTAATCGCGGTAACATAACGGTGGGTAAGGT
CD19	SJ25C1	AHS0030	TAGTAATGTGTTCGTAGCCGGTAATAATCTTCGTGG
CD25	2A3	AHS0026	AGTTGTATGGGTTAGCCGAGAGTAGTGCGTATGATT
CD27	M-T271	AHS0025	TGTCCGGTTTAGCGAATTGGGTTGAGTCACGTAGGT
CD28	L293	AHS0138	TTGTTGAGGATACGATGAAGCGGTTTAAGGGTGTGG
CD45RA	HI100	AHS0009	AAGCGATTGCGAAGGGTTAGTCAGTACGTTATGTTG
CD56	NCAM16	AHS0019	AGAGGTTGAGTCGTAATAATAATCGGAAGGCGTTGG
CD62L	DREG-56	AHS0049	ATGGTAAATATGGGCGAATGCGGGTTGTGCTAAAGT
CD127	HIL-7R-M21	AHS0028	AGTTATTAGGCTCGTAGGTATGTTTAGGTTATCGCG
CD134	ACT35	AHS0013	GGTGTTGGTAAGACGGACGGAGTAGATATTCGAGGT
CD137	4B4-1	AHS0003	TGACAAGCAACGAGCGATACGAAAGGCGAAATTAGT
CD161	HP-3G10	AHS0205	TTTAGGACGATTAGTTGTGCGGCATAGGAGGTGTTC
CD183 (CXCR3)	1C6/CXCR3	AHS0031	AAAGTGTTGGCGTTATGTGTTCGTTAGCGGTGTGGG
CD185 (CXCR5)	RF8B2	AHS0039	AGGAAGGTCGATTGTATAACGCGGCATTGTAACGGC
CD186 (CXCR6)	13B 1E5	AHS0148	GTGGTTGGTTATTCGGACGGTTCTATTGTGAGCGCT
CD196 (CCR6)	11A9	AHS0034	ACGTGTTATGGTGTTGTTCGAATTGTGGTAGTCAGT
CD197 (CCR7)	2-L1-A	AHS0273	AATGTGTGATCGGCAAAGGGTTCTCGGGTTAATATG
CD272	J168-540	AHS0052	GTAGGTTGATAGTCGGCGATAGTGCGGTTGAAAGCT
CD278	DX29	AHS0012	ATAGTCCGCCGTAATCGTTGTGTCGCTGAAAGGGTT
CD279	EH12.1	AHS0014	ATGGTAGTATCACGACGTAGTAGGGTAATTGGCAGT
CD357 (GITR)	V27-580	AHS0104	TCTGTGTGTCGGGTTGAATCGTAGTGAGTTAGCGTG
CD366 (Tim3)	7D3	AHS0016	TAGGTAGTAGTCCCGTATATCCGATCCGTGTTGTTT
HLA-DR	G46-6	AHS0035	TGTTGGTTATTCGTTAGTGCATCCGTTTGGGCGTGG
IgM	G20-127	AHS0198	TTTGGAGGGTAGCTAGTTGCAGTTCGTGGTCGTTTC
IgD	IA6-2	AHS0058	TGAGGGATGTATAGCGAGAATTGCGACCGTAGACTT