Technical Data Sheet

NK-Cell Protein Panel

Product Information

Material Number:	**572434**
Size:	2 Tests
Vol. per Test:	NA
Reactivity:	Tested in Development: Human
Storage Buffer:	Lyophilized powder containing BSA and ≤ 0.25% sodium azide.
Component:	**51-9025299**
Description:	NK-Cell Protein Panel
Size:	1 Test (2 ea)
Vol. per Test:	NA

Description

The BD® OMICS-One NK-Cell Protein Panel consists of 30 different specificities against major NK-Cell markers in a single tube. Designed and optimized to work on the BD Rhapsody™ System, the NK-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 BD Rhapsody™ Enhanced Cell Capture Beads. The 5' PCR handle allows for efficient 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 NK-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 reallocation of sequencing reads to markers expressed at lower levels. With SMART technology, markers low in expression can be quantified without having to do deeper sequencing and incurring high sequencing cost. The two specificities attenuated in the NK-Cell Protein Panel are CD2 and CD31.

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 NK-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. Place 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.
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 follows:

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 BD® AbSeq Ab-Oligo	2.0 × N*	2.6 × N	5.2 × N

BD Biosciences
bdbiosciences.com

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FBS† (catalog number 554656)	70 – (2.0 x N)	91 – (2.6 x N)	182 – (5.2 x N)
Total	**70**	**91**	**182**

For co-labeling with Sample Tags, prepare BD® AbSeq labeling MasterMix for drop-ins as follows:

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 BD® AbSeq Ab-Oligo	2.0 × N*	2.6 × N	5.2 × N
FBS† (catalog number 554656)	120 – (2.0 × N)	156 – (2.6 × N)	312 – (5.2 × N)
Total	**120**	**156**	**312**

* N = number of drop-in antibodies. N = 0 if there are no drop-in antibodies.
† FBS = BD Pharmingen™ Stain Buffer.

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. If 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 NK-Cell Protein Panel solution to make a total volume of 175 µL.
   If 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 NK-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, we recommend 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)
FBS† (catalog number 554656)	20.0	24.0
Fc Block‡ (catalog number 564220)	5.0	6.0
Total	**25.0**	**30.0**

* Sufficient for up to 1 million cells. To block more cells, adjust the volume.
† FBS = BD Pharmingen™ Stain Buffer.
‡ Fc Block = BD Pharmingen™ Human BD Fc Block.

    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 x *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 three 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 substeps 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 follows:
    a. At the protocol section of "Loading cells in BD Rhapsody™ 8-Lane Cartridge", after cell load, incubate the cartridge in the dark at room temperature for 8 minutes.
    b. Place the cartridge on the BD Rhapsody™ HT Xpress and perform the On-Cartridge Wash steps listed as follows:

Material to load	Volume (µL) 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
CD11b	M1/70	AHS0005	ATCGTTATTCGTTGTAGTTCGCCCGGTTTGAGTAGT
CD56	NCAM16.2	AHS0019	AGAGGTTGAGTCGTAATAATAATCGGAAGGCGTTGG
CD38	HIT2	AHS0022	GTCAACGATGGGTAGCGGTAGAAATAACGGAACTGG
CD27	M-T271	AHS0025	TGTCCGGTTTAGCGAATTGGGTTGAGTCACGTAGGT
CD2	RPA-2.10	AHS0029	AAACGTAGATTAGAGCCGGGTATGTCGCAACTGATT
CD16	3G8	AHS0053	TAAATCTAATCGCGGTAACATAACGGTGGGTAAGGT
CD184 (CXCR4)	12G5	AHS0060	CAGTGTTTAGAGCGGGTTGCATATGTCGTTTAGAGG
CD49d	9F10	AHS0063	TAGGGTGACTTAGCGATTGATGCGTATGTTTGGGCG
CD314 (NKG2D)	1D11	AHS0065	TTGAAATGCGATGAGACGTAGAGCGATGTAGGTAGC
CD335 (NKP46)	9E2/NKP46	AHS0068	CAATTTGTTCGCGTTTAGTAGTCGTCGTCTTATGGG
CD54	HA58	AHS0076	AAGAGAATATATGCGTGCGTTGTTAAGGGAATGCGT
CD226	DX11	AHS0079	GAGTTTATGATTCGTTTCTTCGGTAGTTCGTCGCTT
CD94	HP-3D9	AHS0085	GAGGTTAGGATAGGTGTACGGGTCGAGTTGAATTCT
CD336 (NKP44)	p44-8	AHS0090	AATGCAAACGATATCACGAAGGGTAGTACACGACGG
CD49a	SR84	AHS0101	ATGACACGAATGCGACGAGAGGCGAAATAGGTTGGT
CX3CR1	2A9-1	AHS0125	GGGTTCACGAGGTTTAAAGCGGTAGTATAGGATGCC
CD122	Mik-β3	AHS0146	TTAAAGAGATTCGTGGGTATTGGCGCAGTCATTCCT
CD140b (PDGFR)	28D4	AHS0151	GACAACATTTAGGACGTGACGAGAGAGTATAGCTTC
CD248	B1/35	AHS0156	ATCACTTATTTCGTTTGGAGGGTTCGTAGGCGTTGC
CD63	H5C6	AHS0157	TGCAGCGTTAGGACCAAGCGTTTACCGTAGAATATT
CD140a	α-R1	AHS0160	TTACTGACTTTCGGACGTTGGTTACTTAGGGTTATG
CD31 (PECAM1)	WM59	AHS0170	CTAAGGGACGTAATTGAGTTTCGGTGATCGCAGTTT
CD96	6F9	AHS0194	CTAATGTAAGAGCGGACGTTTGGGCACTATATGTTT
CD161 (KLRB1)	HP-3G10	AHS0205	TTTAGGACGATTAGTTGTGCGGCATAGGAGGTGTTC
CD158b (KIR)	DX27	AHS0209	CGTAGGAGGATTTCGTCGATGGGTTTGTTAGCGTTC
CD158e1	DX9	AHS0211	AGGTTCATTGCGGCATTAGGCGTCATATAGTAGGTG
CD337/NKp30	P30-15	AHS0213	GGTAACTGACATGACGGAGCGATAATTTCTGGCGGT
CD3	UCHT1	AHS0231	AGCTAGGTGTTATCGGCAAGTTGTACGGTGAAGTCG
CD329(Siglec-9)	E10-286	AHS0239	CGGGCGCGAAGATAGGATAATAGGTAACGTCAAATG
CD106	51-10C9	AHS0251	TCTGATTTAGCGGGTGGACGTATTATAGTGATTGGC

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
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)
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. 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. Please refer to https://www.bdbiosciences.com/en-us/resources/protocols/single-cell-multiomics for technical 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, see bd.com/patents.