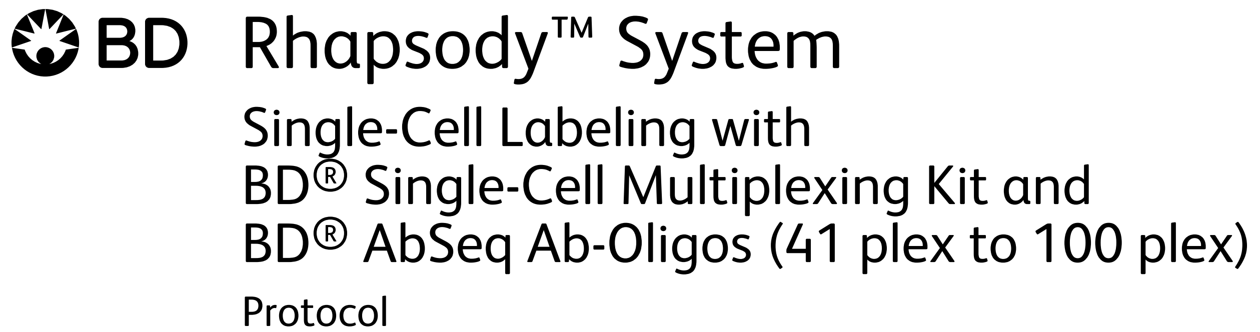


BD Rhapsody™ System

Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (41 plex to 100 plex)

Protocol

23-22354(02)

2022-11

For Research Use Only

Copyrights

No part of this publication may be reproduced, transmitted, transcribed, stored in retrieval systems, or translated into any language or computer language, in any form or by any means: electronic, mechanical, magnetic, optical, chemical, manual, or otherwise, without prior written permission from BD.

The information in this guide is subject to change without notice. BD reserves the right to change its products and services at any time. Although this guide has been prepared with every precaution to ensure accuracy, BD assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information. BD welcomes customer input on corrections and suggestions for improvement.

Trademarks

BD, the BD Logo, BD Rhapsody and Fc Block are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2022 BD. All rights reserved.

For US patents that may apply, see bd.com/patents.

Regulatory information

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

History

Revision	Date	Change made
23-22354-00	2020-05	Initial release.
23-22354(01)	2021-11	Added BD Rhapsody™ Enhanced Cell Capture Beads and part numbers.
23-22354(02)	2022-11	Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers.

Contents

Introduction 4

Workflow 5

Required materials 6

Suggested materials 6

Before you begin 6

Safety information 6

Preparing BD® AbSeq labeling MasterMix 7

Co-labeling single-cell samples with Sample Tags and BD® AbSeq Ab-Oligos 9

Sequential labeling of single-cell samples, first with Sample Tags, then with BD® AbSeq Ab-Oligos 10

Labeling cells with Sample Tags 10

Labeling cells with BD® AbSeq Ab-Oligos 11

Washing labeled cells 12

Troubleshooting 13

Appendix A: Sample Tag sequences 14

Introduction

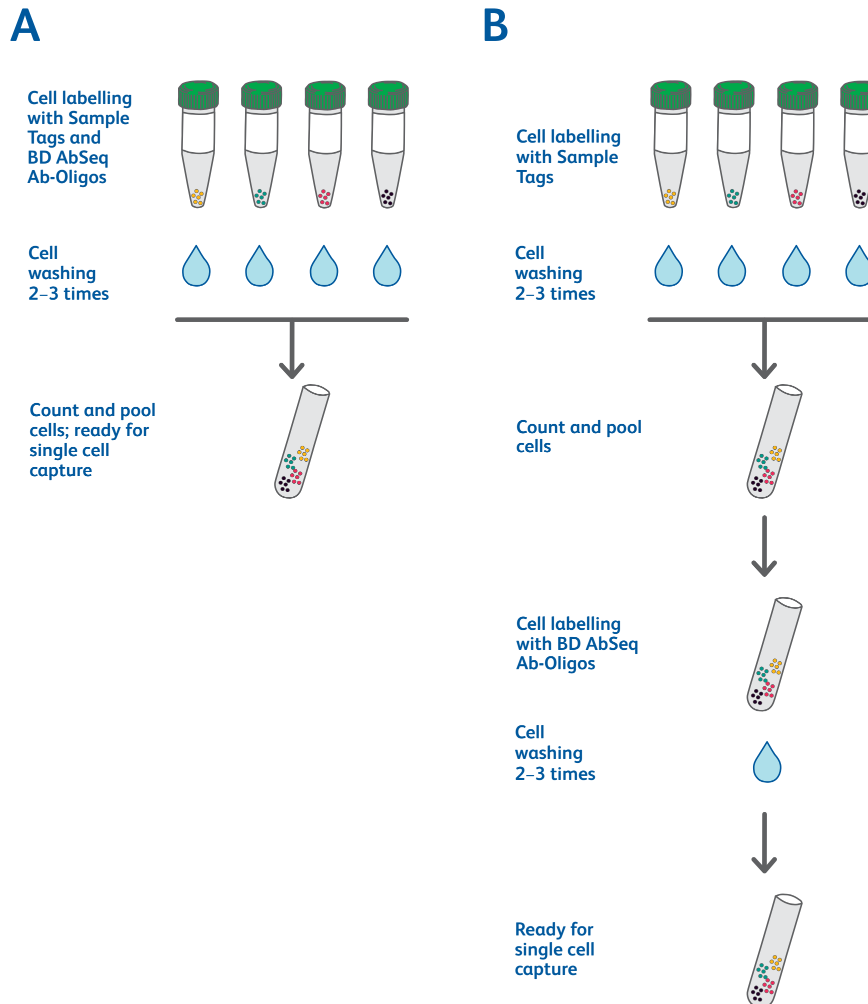
This protocol describes the use of BD® AbSeq Ab-Oligos (antibody-oligonucleotides) with the BD® Human Single-Cell Multiplexing Kit.

The BD® AbSeq Ab-Oligos are used for antigen-expression profiling with BD Rhapsody™ single-cell capture and downstream library preparation. Each BD® AbSeq Ab-Oligo is an oligonucleotide-conjugated antibody that contains an antibody-specific barcode and poly(A)-tail for bead capture, PCR amplification, and library generation. The protocol supports BD® AbSeq labeling of 20,000 to 1 million cells. Up to 100 antibodies can be pooled together per staining reaction.

The BD® Single-Cell Multiplexing Kit utilizes an innovative antibody-oligo technology to provide higher sample throughput for single-cell library preparation. Every antibody-oligo in the kit, referred to as a Sample Tag, has a unique sample barcode conjugated to a human universal antibody. Up to 12 samples can be labeled and pooled prior to single-cell capture with the BD Rhapsody™ Single-Cell Analysis System or other single-cell analysis systems.

Workflow

You can co-label cells with Sample Tags and BD® AbSeq Ab-Oligos in a single tube (A), or you can sequentially label cells with Sample Tags and pool cells before labeling with BD® AbSeq Ab-Oligos (B):



Sequential labeling is more economical than co-labeling, but you will save time by co-labeling. The biological effects of co-labeling versus sequential labeling might be different. These effects might depend on cell type and experimental condition. Consider potential effects in your experimental design.

Required materials

For a complete list of materials, see the appropriate BD Rhapsody™ instrument user guide.

Note: Use only the tubes specified in the protocol. Use of other tubes could lead to sub-optimal results.

Material	Supplier	Catalog no.
20,000-1 million cells	–	–
BD® Stain Buffer (FBS)	BD Biosciences	554656
BD® AbSeq Ab-Oligos[a]	BD Biosciences	Various
BD® Human Single-Cell Multiplexing Kit[a] Or, BD® Mouse Immune Single-Cell Multiplexing Kit[a]	BD Biosciences BD Biosciences	633781 633793
BD Rhapsody™ Enhanced Cartridge Reagent Kit	BD Biosciences	664887
Latch Rack for 500-µL tubes	Thermo Fisher Scientific	4900 or 4890
Falcon® tubes, 5-mL round-bottom, polystyrene test tubes[b]	Corning	352054

a. Avoid storing BD® AbSeq Ab-Oligos or Sample Tags under freezing conditions.

b. Use only the tubes specified in the protocol. Use of other tubes might lead to increased cell loss.

Suggested materials

Material	Supplier	Catalog no.
Human BD Fc Block™ Or, Mouse BD Fc Block™	BD Biosciences BD Biosciences	564220 553142
8-Channel Screw Cap Tube Capper	Thermo Fisher Scientific	4105MAT
Multi-channel pipette	Major supplier	–

Before you begin

- Use low retention filtered pipette tips.
- Prepare a single-cell suspension. See *Preparing Single-Cell Suspensions Protocol*.
- If your biological sample contains red blood cell contamination, red blood cell lysis is required. See *Preparing Single-Cell Suspensions Protocol*.

Safety information

For safety information, see the *BD Rhapsody™ Single-Cell Analysis Instrument User Guide* or the *BD Rhapsody™ Express Single-Cell Analysis System Instrument User Guide*.

Preparing BD® AbSeq labeling MasterMix

We recommend:

- Creating freshly pooled antibodies before each experiment.
- Creating pools with 30% overage to ensure adequate volumes for labeling. The reagents are viscous and can form bubbles easily.
- For high-plex, using an 8-Channel Screw Cap Tube Capper and multi-channel pipette to pipet BD® AbSeq Ab-Oligos into 8-tube strips. Centrifuge the tube strip and pool BD® AbSeq Ab-Oligos into a 1.5-mL tube.

1. Place all tubes of BD® AbSeq Ab-Oligos to be pooled into a Latch Rack for 500-µL tubes. Arrange the tubes so that they can be easily uncapped and re-capped with an 8-Channel Screw Cap Tube Capper and aliquoted with a multi-channel pipette.
2. Centrifuge the BD® AbSeq Ab-Oligos tubes in the Latch Rack in a tabletop centrifuge with a plate adapter at 400 × *g* for 30 seconds and place on ice.
3. Follow one of two workflows to label cells with Sample Tags and BD® AbSeq Ab-Oligos:
   - Co-labeling single-cell samples with Sample Tags and BD® AbSeq Ab-Oligos. Ensures each sample is labeled independently with Sample Tags and Ab-Oligos.
   - Sequential labeling of single-cell samples, first with Sample Tags, then with BD® AbSeq Ab-Oligos. After labeling with Sample Tags, samples are pooled and stained together with BD® AbSeq Ab-Oligos. This method provides BD® AbSeq reagent cost savings.
4. In the pre-amplification workspace, pipet the reagents into a new 1.5-mL LoBind tube on ice.

   BD® AbSeq labeling MasterMix

   For 87 plex and below, use the following volumes:

   a. Without optional Fc Block™ – 200 µL AbSeq labeling MasterMix
   b. With optional Fc Block™ – 175 µL AbSeq labeling MasterMix (25 µL Fc Block™ MasterMix will be prepared and added separately [see Fc Block™ MasterMix for information on making Fc Block™ MasterMix])

   For 88 plex and above, use the following volumes:

   a. Without optional Fc Block™ – 200 µL AbSeq labeling MasterMix
   b. With optional Fc Block™ – 200 µL AbSeq labeling MasterMix (25 µL Fc Block™ MasterMix will be prepared and added separately [see Fc Block™ MasterMix for information on making Fc Block™ MasterMix])

   Note: When using Fc Block™ with >87 plex, the total volume will be over 200 µL. Incubation times longer than 30 minutes may increase sensitivity.

   See the following examples.

Component	For 1 sample (µL)	For 1 sample + 30% overage (µL)	For 2 samples + 30% overage (µL)
Per BD® AbSeq Ab-Oligo	2.0	2.6	5.2
Total	**2.0 * *N***	**2.6 * *N***	**5.2 * *N***
BD® Stain Buffer (FBS)	200 – (2.0 * *N*)	260 – (2.6 * *N*)	520 – (5.2 * *N*)
Total	**200.0**	**260**	**520**
(*N* = number of antibodies)			

Examples of different pools of BD® AbSeq Ab-Oligos are described in the following table.

Component	For 1 sample (µL)	For 1 sample + 30% overage (µL)	For 2 samples + 30% overage (µL)
60-plex BD® AbSeq labeling without Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (120.0 total)	2.6 (156.0 total)	5.2 (312.0 total)
BD® Stain Buffer (FBS)	80	104	208
Total	**200**	**260**	**520**
60-plex BD® AbSeq labeling with Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (120.0 total)	2.6 (156.0 total)	5.2 (312.0 total)
BD® Stain Buffer (FBS)	55	71.5	143
Total	**175**	**227.5**	**455**
90-plex BD® AbSeq labeling with or without Fc Block™			
Per BD® AbSeq Ab-Oligo	2.0 (180.0 total)	2.6 (234.0 total)	5.2 (468.0 total)
BD® Stain Buffer (FBS)	20	26	52
Total	**200**	**260**	**520**

5 Pipet-mix the BD® AbSeq labeling MasterMix and place back on ice.

Co-labeling single-cell samples with Sample Tags and BD® AbSeq Ab-Oligos

1 To each Sample Tag tube containing 20 µL of Sample Tag, add 200 µL BD® AbSeq labeling MasterMix (175 µL if <87 plex and Fc Block™ is being performed). This is the “AbSeq/Sample Tag labeling MasterMix.”

2 Centrifuge the cells at 400 × *g* for 5 minutes.

3 (Optional) For samples containing myeloid and B lymphocytes, we recommend blocking non-specific Fc Receptor–mediated false-positive signals with Human BD Fc Block™.

To perform blocking:

a Pipet the reagents into a new 1.5-mL LoBind tube on ice:

Fc Block™ MasterMix

Component	For 1 sample (µL)[a]	For 1 sample + 20% overage (µL)
BD® Stain Buffer (FBS)	20.0	24.0
Human BD Fc Block™ or Mouse BD Fc Block™	5.0	6.0
Total	**25.0**	**30.0**

a. Sufficient for ≤1 × 10^6 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 (15–25 °C) for 10 minutes.

f After Fc Block™, add BD® AbSeq/Sample Tag labeling MasterMix into the cell suspension. Pipet-mix and proceed to **step 5**.

4 Remove the supernatant from the cells without disturbing the pellet and resuspend with the BD® AbSeq/Sample Tag labeling MasterMix. Pipet-mix.

5 Transfer the cells and the labeling MasterMix into a new 5-mL polystyrene Falcon® tube.

6 Incubate on ice for 30–60 minutes.

Note: If the staining volume exceeds 200 µL, incubation times longer than 30 minutes may increase sensitivity.

Caution: Aqueous buffered solution (Sample Tag) contains BSA and 0.1% sodium azide. 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.

7 Proceed to Washing labeled cells.

Sequential labeling of single-cell samples, first with Sample Tags, then with BD® AbSeq Ab-Oligos

Labeling cells with Sample Tags

1 Resuspend 20,000–1 million cells in 190 µL BD® Stain Buffer (FBS).

2 Briefly centrifuge the Sample Tag tubes to collect the contents at the bottom.

3 To each Sample Tag tube containing 20 µL of Sample Tag, transfer 180 µL of cell suspension. Pipet-mix.

Caution: Aqueous buffered solution (Sample Tag) contains BSA and 0.1% sodium azide. 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.

4 Incubate at room temperature (15–25 °C) for 20 minutes.

5 Transfer each labeled cell suspension to a 5-mL polystyrene Falcon® tube.

6 Add 2 mL of BD® Stain Buffer to the labeled cells and pipet-mix.

7 Centrifuge each tube at 400 × *g* for 5 minutes.

8 Uncap each tube and invert to decant the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from the tube rim.

9 Add 2 mL of BD® Stain Buffer to each tube and pipet-mix to resuspend.

10 Centrifuge at 400 × *g* for 5 minutes.

11 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.

12 (Optional) Repeat **step 9 through step 11** once more for a total of two washes.

13 Count and pool cells to desired ratios. For subsequent BD® AbSeq staining ensure the total number of pooled cells is within the range of 20,000–1 million cells.

Labeling cells with BD® AbSeq Ab-Oligos

1 Centrifuge the cells at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.

2 (Optional) For samples containing myeloid and B lymphocytes, we recommend blocking non-specific Fc Receptor–mediated false-positive signals with Human BD Fc Block™ or Mouse BD Fc Block™, as appropriate.

To perform blocking:

a Pipet the reagents into a new 1.5-mL LoBind tube on ice:

Fc Block™ MasterMix

Component	For 1 sample (µL)[a]	For 1 sample + 20% overage (µL)
BD® Stain Buffer (FBS)	20.0	24.0
Human BD Fc Block™ or Mouse BD Fc Block™	5.0	6.0
Total	**25.0**	**30.0**

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

b Pipet-mix the Fc Block™ MasterMix and briefly centrifuge. Place on ice.

c Discard 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 (15–25 °C) for 10 minutes.

f After Fc Block™, add 175 µL (87 plex and below) or 200 µL (88 plex and above) of BD® AbSeq labeling MasterMix into the cell suspension. Pipet-mix and proceed to **step 4**.

3 Discard the supernatant from the cells without disturbing the pellet, and resuspend with 200 µL of BD® AbSeq labeling MasterMix. Pipet-mix.

4 Transfer the cells and AbSeq labeling MasterMix (200–225 µL) into a new 5-mL polystyrene Falcon® tube.

5 Incubate on ice for 30–60 minutes.

Note: If the staining volume exceeds 200 µL, incubation times longer than 30 minutes may increase sensitivity.

Washing labeled cells

Note: Sufficient post-labeling washing is important for reducing noise that comes from residual unbound Ab-Oligos being captured onto 3' capture beads during single-cell capture. However, some cell loss occurs with each additional wash. You can choose to perform more or fewer washes depending on the cell abundance.

1. Add 3 mL of BD® Stain Buffer to labeled cells and pipet-mix for dilution.
2. Centrifuge each tube at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.
3. Uncap each tube, and invert to decant the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.
4. Add 3 mL BD® Stain Buffer to each tube and resuspend by pipet-mixing for the first wash.
5. Centrifuge at 400 × *g* for 5 minutes, or at an appropriate speed to pellet the cells.
6. Uncap each tube and invert to decant the supernatant into biohazardous waste. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from the tube rim.
7. (Optional) Repeat **step 4** through **step 6** once or twice more for a total of two to three washes.
8. Resuspend the pellet in 620 µL cold Sample Buffer from the BD Rhapsody™ Enhanced Cartridge Reagent Kit. Perform viability staining and count the cells using the appropriate single-cell capture and cDNA synthesis protocol.

   Note: For low-abundance samples (<20,000), resuspend the cells in 200 µL of cold BD® Sample Buffer. For other 3' single-cell capture platforms, resuspend in recommended buffer and volume according to manufacturer.

9. Place the tube on ice, and proceed to single-cell capture. See the *Single-Cell Analysis Workflow with BD Rhapsody™ Systems* to find the appropriate protocol to follow.

Troubleshooting

Observation	Possible causes	Recommended solutions
Do not have the recommended buffer for labeling with Sample Tag or BD® AbSeq Ab-Oligos.	Various.	Labeling with Sample Tags and BD® AbSeq Ab-Oligos has been optimized in BD® Stain Buffer (FBS). Use of other staining buffers could result in less than optimal staining.
Total stain volume exceeds 245 µL.	Co-Staining with Sample Tags with ≥100 BD® AbSeq Ab-Oligos.	Co-Staining Sample Tags with ≥100 BD® AbSeq Ab-Oligos requires that no BD® Stain Buffer (FBS) is used in order to keep the staining volume between 220–245 µL. Co-Staining Sample Tags with ≥100 BD® AbSeq Ab-Oligos results in >220–245 µL (Fc Block™) stain volume may require incubation up to 60 minutes on ice for optimal results.
Cells require labeling with Sample Tags and/or BD® AbSeq Ab-Oligos at a different temperature.	Physiological requirement.	Protocols for Sample Tags and/or BD® AbSeq Ab-Oligo labeling have been optimized for staining on ice. Use of other staining temperatures has not been tested and requires user optimization. For certain cytokine receptors, staining at room temperature can increase the sensitivity. However, incubation at room temperature for long periods of time might negatively impact the cell viability and RNA quality.
Cell loss.	Wrong tube used in washes.	Use Falcon® polystyrene flow tubes and centrifuge cells using a benchtop centrifuge with swing bucket rotor. This centrifugation method reduces cell loss.
	Excessive washing or loss during washing.	We recommend washing cells at least twice before loading onto the BD Rhapsody™ cartridge to decrease noise caused by unbound Ab-Oligos. Fewer washes can cause higher levels of noise. Therefore, for certain cell types, user optimization is required for the washing step to minimize cell loss and to avoid high levels of noise.
Cell loss during sorting.	Various.	• Sort more cells than needed for cartridge loading. • Label Sample Tags and/or BD® AbSeq and fluorescent antibody together to reduce cell loss during multiple washing steps, and proceed to cartridge loading right after sorting.

Appendix A: Sample Tag sequences

Each Human Sample Tag is a human universal antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence:

GTTGTCAAGATGCTACCGTTCAGAG[Sample Tag sequence]AAAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1—Human	ATTCAAGGGCAGCCGCGTCACGATTGGATACGACTGTTGGACCGG
Sample Tag 2—Human	TGGATGGGATAAGTGCGTGATGGACCGAAGGGACCTCGTGGCCGG
Sample Tag 3—Human	CGGCTCGTGCTGCGTCGTCTCAAGTCCAGAAACTCCGTGTATCCT
Sample Tag 4—Human	ATTGGGAGGCTTTCGTACCGCTGCCGCCACCAGGTGATACCCGCT
Sample Tag 5—Human	CTCCCTGGTGTTCAATACCCGATGTGGTGGGCAGAATGTGGCTGG
Sample Tag 6—Human	TTACCCGCAGGAAGACGTATACCCCTCGTGCCAGGCGACCAATGC
Sample Tag 7—Human	TGTCTACGTCGGACCGCAAGAAGTGAGTCAGAGGCTGCACGCTGT
Sample Tag 8—Human	CCCCACCAGGTTGCTTTGTCGGACGAGCCCGCACAGCGCTAGGAT
Sample Tag 9—Human	GTGATCCGCGCAGGCACACATACCGACTCAGATGGGTTGTCCAGG
Sample Tag 10—Human	GCAGCCGGCGTCGTACGAGGCACAGCGGAGACTAGATGAGGCCCC
Sample Tag 11—Human	CGCGTCCAATTTCCGAAGCCCCGCCCTAGGAGTTCCCCTGCGTGC
Sample Tag 12—Human	GCCCATTCATTGCACCCGCCAGTGATCGACCCTAGTGGAGCTAAG

Each Mouse Immune Sample Tag is an Anti-Mouse CD45, Clone 30-F11 antibody conjugated with a unique oligonucleotide sequence to allow for sample identification. Each Sample Tag has common 5' and 3' ends and the Sample Tag sequence:

GTTGTCAAGATGCTACCGTTCAGAG[Sample Tag sequence]AAAAAAAAAAAAAAAAAAAAAAAAA

Sample Tag	Sample Tag sequence
Sample Tag 1—Mouse Immune	AAGAGTCGACTGCCATGTCCCCTCCGCGGGTCCGTGCCCCCCAAG
Sample Tag 2—Mouse Immune	ACCGATTAGGTGCGAGGCGCTATAGTCGTACGTCGTTGCCGTGCC
Sample Tag 3—Mouse Immune	AGGAGGCCCCGCGTGAGAGTGATCAATCCAGGATACATTCCCGTC
Sample Tag 4—Mouse Immune	TTAACCGAGGCGTGAGTTTGGAGCGTACCGGCTTTGCGCAGGGCT
Sample Tag 5—Mouse Immune	GGCAAGGTGTCACATTGGGCTACCGCGGGAGGTCGACCAGATCCT
Sample Tag 6—Mouse Immune	GCGGGCACAGCGGCTAGGGTGTTCCGGGTGGACCATGGTTCAGGC
Sample Tag 7—Mouse Immune	ACCGGAGGCGTGTGTACGTGCGTTTCGAATTCCTGTAAGCCCACC
Sample Tag 8—Mouse Immune	TCGCTGCCGTGCTTCATTGTCGCCGTTCTAACCTCCGATGTCTCG
Sample Tag 9—Mouse Immune	GCCTACCCGCTATGCTCGTCGGCTGGTTAGAGTTTACTGCACGCC
Sample Tag 10—Mouse Immune	TCCCATTCGAATCACGAGGCCGGGTGCGTTCTCCTATGCAATCCC
Sample Tag 11—Mouse Immune	GGTTGGCTCAGAGGCCCCAGGCTGCGGACGTCGTCGGACTCGCGT
Sample Tag 12—Mouse Immune	CTGGGTGCCTGGTCGGGTTACGTCGGCCCTCGGGTCGCGAAGGTC

Becton, Dickinson and Company
BD Biosciences
2350 Qume Drive
San Jose, California 95131 USA

bdbiosciences.com
scomix@bdscomix.bd.com