# BD Rhapsody™ System

## Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (1 plex to 40 plex)

Protocol

23-21339(02)
2022-11

For Research Use Only

### Regulatory information

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

### History

| Revision | Date | Change made |
|---|---|---|
| Doc ID 214419 Rev. 1.0 | 2018-07 | Initial release. |
| Doc ID 214419 Rev. 2.0<br>23-21339-00 | 2019-02 | Added Mouse Immune Sample Tag sequences to Appendix A: Sequence Information. |
| Doc ID: 214419 Rev. 3.0<br>23-21339(01) | 2021-11 | Added BD Rhapsody™ Enhanced Cell Capture Beads and part numbers. |
| 23-21339(02) | 2022-11 | Updated for BD Rhapsody™ Enhanced Cell Capture Beads v2.0. Removed part numbers. |

# Contents

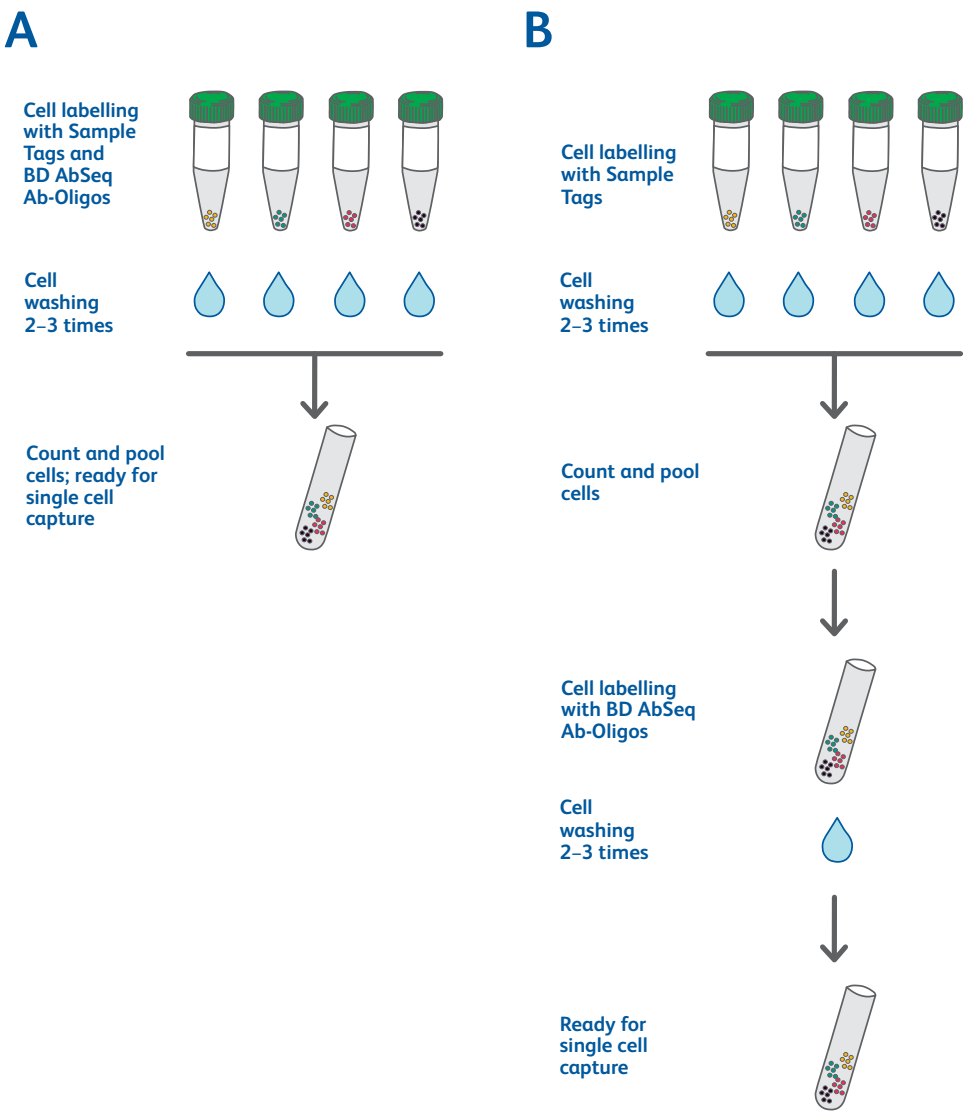
# Introduction

This protocol describes use of BD® AbSeq Ab-Oligos (antibody-oligonucleotides) with the BD® Human Single-Cell Multiplexing Kit or the BD® Mouse Immune 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-Oligos 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 40 antibodies can be pooled together per staining reaction. For greater than 40 antibodies, refer to the *BD Rhapsody™ System Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos (41 plex to 100 plex) Single-Cell Protocol*.

The BD® Single-Cell Multiplexing Kits utilize an innovative antibody-oligo technology to provide higher sample throughput for single-cell library preparation. Every antibody-oligo in the BD® Human Single-Cell Multiplexing Kit, referred to as a Sample Tag, has a unique sample oligo barcode to a human universal antibody and every Sample Tag in the BD® Mouse Immune Single-Cell Multiplexing Kit is conjugated to an Anti-Mouse CD45, Clone 30-F11 antibody. Up to 12 samples can be labeled and pooled prior to single-cell capture with the BD Rhapsody™ Single-Cell Analysis System.

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

| 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]<br>Or,<br>BD® Mouse Immune Single-Cell Multiplexing Kit[a] | BD Biosciences | 633781<br><br>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.

For a complete list of materials, see appropriate instrument user guide.

# Suggested materials

| Material | Supplier | Catalog no. |
|---|---|---|
| Human BD Fc Block™<br>Or,<br>Mouse BD Fc Block™ | BD Biosciences<br><br>BD Biosciences | 564220<br><br>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 Ab-Oligos

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 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 tube strip and pool BD® AbSeq Ab-Oligos into a 1.5-mL LoBind tube.

1. Place all 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 BD® AbSeq Ab-Oligos in the Latch Rack in a tabletop centrifuge with a plate adapter tubes 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-cells with BD® AbSeq Ab-Oligos and Sample Tags
   - Sequentially labeling single-cells with Sample Tags and BD® AbSeq Ab-Oligos

# Co-labeling single-cells with BD® AbSeq Ab-Oligos and Sample Tags

**1** In pre-amplification workspace, pipet reagents into a new 1.5-mL LoBind Tube on ice:

**2X BD® AbSeq labeling MasterMix for co-labeling workflow**

| 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 |
| BD Pharmingen™ Stain Buffer (FBS)<br>(*N* = no. antibodies) | 80 – (2.0 × *N*) | 104 – (2.6 × *N*) | 208 – (5.2 × *N*) |
| **Total** | **80.0** | **104.0** | **208.0** |

**Examples**

| Component | 1 sample (µL) | 1 sample + 30% overage (µL) | 2 samples + 30% overage (µL) |
|---|---|---|---|
| **10-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (20.0 total) | 2.6 (26.0 total) | 5.2 (52.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 60.0 | 78.0 | 156.0 |
| **20-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (40.0 total) | 2.6 (52.0 total) | 5.2 (104.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 40.0 | 52.0 | 104.0 |
| **40-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (80.0 total) | 2.6 (104.0 total) | 5.2 (208.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 0.0 | 0.0 | 0.0 |

**2** Pipet-mix the 2X AbSeq labeling MasterMix, and place back on ice.

**3** To each Sample Tag tube containing 20 µL of Sample Tag, add 80.0 µL 2X AbSeq labeling MasterMix.

**4** Pipet-mix, and place on ice.

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

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

To perform blocking:

**a** Pipet 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 Pharmingen™ Stain Buffer (FBS) | 95.0 | 114.0 |
| BD Pharmingen™ Human BD Fc Block™ or Mouse BD Fc Block™ | 5.0 | 6.0 |
| **Total** | **100.0** | **120.0** |

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

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

**c** Discard supernatant from cells without disturbing pellet.

**d** Resuspend cells in 110 µL Fc Block™ MasterMix.

**e** Incubate cells at room temperature (15–25 °C) for 10 minutes.

**f** After Fc Block™, proceed to **step 8**.

**7** Discard supernatant from cells without disturbing pellet and resuspend each sample in 110 µL BD® Stain Buffer. Pipet-mix.

**8** Pipet 100 µL of each cell suspension into the corresponding Sample Tag tube that contains 2X BD® AbSeq and Sample Tag labeling master mix for co-labeling workflow. 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.

**9** Incubate on ice for 30–60 minutes.

**10** Proceed to Washing the labeled cells.

# Sequentially labeling single-cells with Sample Tags and BD® AbSeq Ab-Oligos

## Labeling with Sample Tags

1 Resuspend 20,000–1 million cells in 200 µL BD® Stain Buffer (FBS).

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

3 For each sample, transfer 180 µL cell suspension to a Sample Tag tube. 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. Add 2 mL BD Pharmingen™ Stain Buffer to labelled cells and pipet-mix.

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

7 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.

8 Add 2 mL BD® Stain Buffer to each tube and resuspend by pipet-mixing.

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

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

11 (Optional) Repeat **steps 8–10** once more for a total of 3 washes.

12 Resuspend pellet in an appropriate volume for counting. If using the BD Rhapsody™ Scanner, resuspend the pellet in 620 µL cold BD® Sample Buffer from the BD Rhapsody™ Enhanced Cartridge Reagent Kit. Perform viability staining and count cell using the appropriate single-cell capture and cDNA synthesis protocol.

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

**Note:** For low-abundance samples (<20,000), resuspend the cells in 200 µL of cold BD® Sample Buffer.

**We recommend pooling more cells (up to one million cells) than you want to be captured in the BD Rhapsody™ Cartridge, because there can be cell loss during BD® AbSeq labeling and washing.**

## Labeling with BD® AbSeq Ab-Oligos

**1** In pre-amplification workspace, pipet reagents into a new 1.5-mL LoBind Tube on ice:

**2X BD® AbSeq labeling MasterMix for sequential labeling workflow**

| 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 |
| BD Pharmingen™ Stain Buffer (FBS)<br>(*N* = no. antibodies) | 100 – (2.0 × *N*) | 130 – (2.6 × *N*) | 260 – (5.2 × *N*) |
| **Total** | **100.0** | **130.0** | **260.0** |

**Examples**

| Component | 1 sample (µL) | 1 sample + 30% overage (µL) | 2 samples + 30% overage (µL) |
|---|---|---|---|
| **10-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (20.0 total) | 2.6 (26.0 total) | 5.2 (52.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 80.0 | 104.0 | 208.0 |
| **20-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (40.0 total) | 2.6 (52.0 total) | 5.2 (104.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 60.0 | 78.0 | 156.0 |
| **40-plex BD® AbSeq labeling** | | | |
| Per BD® AbSeq Ab-Oligo | 2.0 (80.0 total) | 2.6 (104.0 total) | 5.2 (208.0 total) |
| BD Pharmingen™ Stain Buffer (FBS) | 20.0 | 26.0 | 52.0 |

**2** Pipet-mix the 2X AbSeq labeling master mix, and place back on ice.

**3** Centrifuge cells at 400 × *g* for 5 minutes.

**4** (Optional) For samples containing myeloid and B lymphocytes, we recommend blocking non-specific Fc Receptor-mediated false-positive signal with Human BD Fc Block™ or Mouse BD Fc Block™.

To perform blocking:

**a** Pipet 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 Pharmingen™ Stain Buffer (FBS) | 95.0 | 114.0 |
| BD Pharmingen™<br>Human BD Fc Block™ or<br>Mouse BD Fc Block™ | 5.0 | 6.0 |
| **Total** | **100.0** | **120.0** |

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

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

c Discard supernatant from cells without disturbing pellet.

d Resuspend cells in 110 µL Fc Block™ MasterMix.

e Incubate cells at room temperature (15–25 °C) for 10 minutes.

f After Fc Block™, proceed to **step 6**.

5 Discard supernatant without disturbing pellet, and resuspend in 110 µL BD® Stain Buffer. Pipet-mix.

6 In new 5 mL polystyrene Falcon® tube, combine 100 µL pooled cell suspension labelled with Sample Tags and 100 µL 2X BD® AbSeq labeling master mix for sequential labeling workflow. Pipet-mix.

7 Incubate on ice for 30–60 minutes.

8 Proceed to Washing the labeled cells.

# Washing the labeled cells

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

1 Transfer each labeled cell suspension to a 5-mL polystyrene Falcon® tube, if the cells are in a different tube type. Add 2 mL BD Pharmingen™ Stain Buffer to labeled cells and pipet-mix.

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 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 2 mL BD® Stain Buffer to each tube, and resuspend by pipet-mixing.

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

7 (Optional) Repeat **steps 4–6** once more for a total of 3 washes.

8 Resuspend the pellet in 620 µL cold Sample Buffer. Perform viability staining and count cell 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 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 Tags or BD® AbSeq Ab-Oligos. | BD Pharmingen™ Stain Buffer not used. | Labeling with Sample Tags and BD® AbSeq Ab-Oligos is optimal in BD® Stain Buffer (FBS). Label Sample Tags and BD® AbSeq Ab-Oligos in BD Pharmingen™ Stain Buffer (FBS). |
| Cells require labeling with Sample Tags and/or BD® AbSeq Ab-Oligos at a different temperature. | Physiological requirement | Use protocols for Sample Tag and/or BD® AbSeq Ab-Oligos labeling that have been optimized for the specific sample type. |
| Accidentally resuspended cells in BD® Stain Buffer (FBS) rather than Sample Buffer before cell counts. | Various. | We recommend centrifuging the samples and resuspending the cells in Sample Buffer after labeling with Sample Tags. This ensures optimal performance of cell loading in the BD Rhapsody™ Cartridge. |
| 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. |
| Cell loss after sorting. | Various. | • Sort more cells than needed for cartridge loading.<br>• Sort cells into 5 mL polystyrene Falcon® tube. Use the same 5 mL polystyrene Falcon® tube that was used for sorting for cell labelling by following these steps:<br>1. Prepare:<br>– For co-labeling: Pipet 100 µL into each Sample Tag tube containing 20 µL Sample Tag and 80 µL 2X BD® AbSeq labeling master mix.<br>– For sequential labeling: Pipet 180 µL BD Pharmingen™ Stain Buffer into each Sample Tag tube containing 20 µL Sample Tag.<br>2. Pipet-mix, and place on ice.<br>3. Sort cells into a 5 mL polystyrene Falcon® tube.<br>4. Centrifuge the sorted cell suspension at 400 × *g* for 5 minutes.<br>5. Uncap the tube and invert to decant supernatant into biohazardous waste.<br>6. Keep the tube inverted and gently blot on a lint-free wiper to remove residual supernatant from tube rim.<br>7. Resuspend cell pellet with the 1X cell labeling master mix (**step 1**), and proceed with cell labeling. |

# 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]AAAAAAAAAAAAAAAAAAAAAAAA

| 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]AAAAAAAAAAAAAAAAAAAAAAAA

| 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 |

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