Co-staining cells with fluorescent antibodies and BD Oligo-Conjugated Antibodies

Capture of isolated single cells on the BD Rhapsody™ Single-Cell Analysis System often requires initially sorting cells to enrich specific subpopulations and to identify rare cells. Here, we address how to co-stain cells with the same clone of both fluorescent antibodies and oligonucleotide-conjugated antibodies (BD® Single-Cell Multiplexing Kit or BD® AbSeq Ab-Oligos).

Recommendations

- Co-stain cells with fluorescent and oligonucleotide-conjugated antibodies (BD® Single-Cell Multiplexing Kit or BD® AbSeq Ab-Oligos) before cell sorting. This can (i) reduce background noise from unbound BD® AbSeq Antibodies and (ii) minimize cell loss from washing that may be more severe with low cell numbers after sorting.
- Use BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to resuspend and wash cells.
- If more than two BD Horizon Brilliant™ Fluorescent Antibodies are present in the sorting panel, use BD Horizon™ Brilliant Stain Buffer Plus (Cat. No. 566385) to reduce dye-to-dye interaction.
- If the same antibody clone is used in both fluorescent and oligonucleotide-conjugated versions:
  - First, adjust the volume or concentration of the antibodies to ensure that equal microgram (µg) quantities are used
  - Second, co-stain cells with these antibodies for 10 minutes on ice, then add the remaining desired antibodies to the cell suspension as described.
- If the specificity for all antibodies used are unique, they can be combined together and cells can be co-stained in a single step. For each antibody, use the recommended volume per test size (e.g., 20 µL of Sample Tag antibody or 2 µL of BD AbSeq Antibody per 1 million cells) established in other BD protocols.
  - For BD® AbSeq Antibodies, refer to *Single Cell Labeling with BD® AbSeq Ab-Oligos*
  - For Sample Tag antibodies (1-plex to 40-plex), refer to *Single Cell Labeling with the BD® Single-Cell Multiplexing Kits and AbSeq Ab-Oligos*
  - For Sample Tag antibodies (41-plex to 100-plex), refer to *Single-Cell Labeling with BD® Single-Cell Multiplexing Kit and BD® AbSeq Ab-Oligos*
  - For fluorescent antibodies, visit us online at **bdbiosciences.com**

    Note: Cell staining protocols are optimized for up to 1 million cells. Higher number of cells used may result in lower binding of antibodies.
- The final staining volume of all antibodies and cell suspension should be 200 µL to ensure best performance of BD® AbSeq Ab-Oligos and BD® Single-Cell Multiplexing Kit.

Co-Staining Workflows

Figure 1A. Same Clone:
Fluorescent Antibody and Ab-Oligo

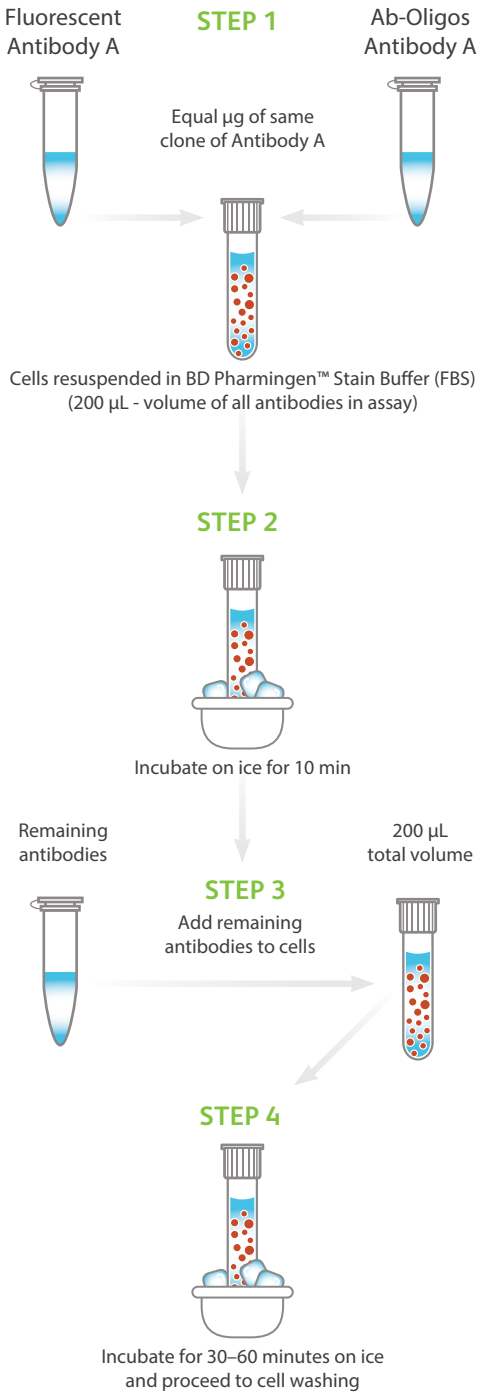

Figure 1B. Unique Specificities Only:
Fluorescent Antibody and Ab-Oligos

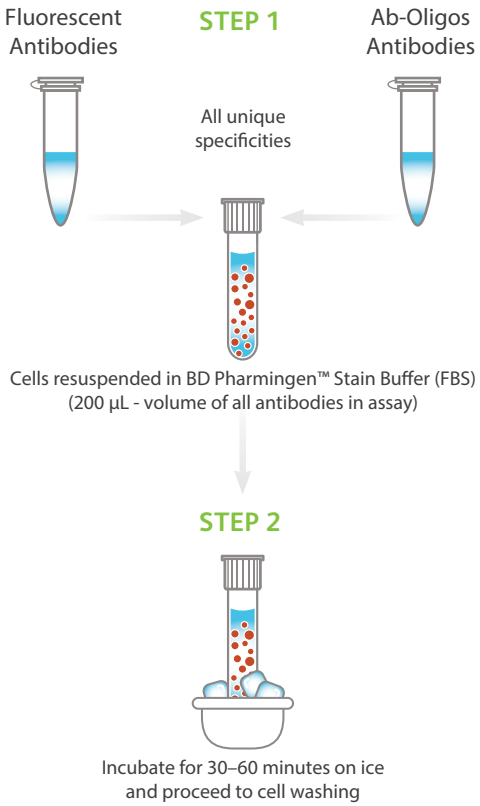

Figure 1. Co-staining workflows
A. Same-clone antibody co-staining: (Step 1) To cells suspended in BD Pharmingen™ Stain Buffer (FBS), add equal µg amounts of fluorescent and oligonucleotide-conjugated antibodies (BD® AbSeq Ab-Oligos or BD® Single-Cell Multiplexing Kit) that share the same clone. **(Step 2)** Incubate antibodies and cell mixture on ice for 10 minutes. **(Step 3)** Add the remaining fluorescent and oligonucleotide-conjugated antibodies to the mixture for a final volume of 200 µL. **(Step 4)** Incubate entire antibody and cell mixture on ice for 30 to 60 minutes and then proceed to cell washing. **B. Co-staining cells with antibodies having unique specificities: (Step 1)** In a single step, combine all fluorescent and oligonucleotide-conjugated antibodies (BD AbSeq Ab-Oligos or BD® Single-Cell Multiplexing Kit) that have unique specificities together with cells suspended in BD Pharmingen™ Stain Buffer (FBS) for a final volume of 200 µL. **(Step 2)** Incubate entire antibody and cell mixture on ice for 30 to 60 minutes and then proceed to cell washing.

Co-staining procedure

1. If there are no overlapping specificities between the fluorescent and oligonucleotide-conjugated antibodies, proceed to step 3.
2. If using the same clone in both fluorescent and oligonucleotide-conjugated versions of the antibody:
   a. Determine the concentration of the fluorescent antibody and the oligonucleotide-conjugated antibody using the following:
      i. For the fluorescent antibody, visit us online at **regdocs.bd.com/regdocs/qcinfo.** Use the catalog number and lot number to find the correct concentration.
      ii. For the oligonucleotide-tagged antibody, contact BD customer support at **scomix@bdscomix.bd.com.**
   b. Determine the appropriate amount of the fluorescent antibody to use so it equals the µg quantity of the oligonucleotide-conjugated antibody.
3. Calculate the total volume of all antibodies to be used in the assay, including adjusted volumes of the same clone from step 2 (if applicable) and volumes for antibodies with different specificities.
4. Subtract the value calculated in step 3 from 200 µL to obtain the volume of BD Pharmingen™ Stain Buffer (FBS) that will be used during staining. (See Table 1 below)
5. Suspend cells in the volume of BD Pharmingen™ Stain Buffer (FBS) calculated in step 4.

   Note: For samples containing myeloid and B lymphocytes, BD recommends blocking non-specific Fc Receptor mediated false-positive with BD Fc Block™ Buffer (human cell, Cat. No 564220; mouse cell, Cat. No. 553142) before staining. For human cells, replace 5 µL of stain buffer with human BD Fc Block™ Buffer and incubate cells for 10 minutes at room temperature. For mouse cells, place 2 uL of stain buffer with mouse BD Fc Block™ Buffer and incubate cells for 5 minutes at 4 °C.
6. If you are not using a shared clone, proceed directly to step 7. If using a shared clone:
   a. Add the appropriate volume of the shared-clone for the fluorescent and oligonucleotide-conjugated antibodies (BD® Single-Cell Multiplexing Kit or BD AbSeq Ab-Oligos).
   b. Pipet-mix and incubate on ice for 10 minutes.
7. Add the remaining fluorescent and/or BD AbSeq Antibodies to attain a final volume of 200 µL.
8. Pipet-mix and incubate the mixture for 30–60 minutes on ice.
9. Transfer labeled cell suspension to a 5 mL polystyrene Falcon™ Tube (Corning™ Cat. No. 352054) if cells are in a different tube type.
10. Add 2 mL BD Pharmingen™ Stain Buffer (FBS) to labeled cells and pipet-mix.
11. Centrifuge tube at 400 x *g* for 5 minutes.
12. Decant supernatant and keep tube inverted. Gently blot inverted tube on lint-free wipe to remove residual liquid from the rim of the tube.

    Note: Do not tilt and decant the tube multiple times to get rid of fluid as this may dislodge cells from the tube.
13. Repeat steps 10–12 once or twice more for a total of 2 or 3 washes.
14. Resuspend cell pellet in BD FACS™ Pre-Sort Buffer (Cat. No. 563503) and proceed with flow-sorting workflow.
15. After sorting, proceed with the protocol outlined in *Single Cell Capture and cDNA Synthesis with the BD Rhapsody Single-Cell Analysis System.*

Component	Volume for one sample
All antibodies	**55 µL** (10 µL + 25 µL + 20 µL)
Shared clone of fluorescent and BD® AbSeq Ab-Oligo (equal µg quantities)	**10 µL** (8 µL flow antibody + 2 µL BD AbSeq Ab-Oligos)
5-plex fluorescent antibodies (unique specificities)	**25 µL** (5 x 5 µL)
10-plex BD® AbSeq Antibodies (unique specificities)	**20 µL** (10 x 2 µL)
Cells suspended in BD Pharmingen™ Stain Buffer (FBS)	**145 µL** (200–55 µL)
Total Volume	**200 µL**

Table 1. Cell labeling example
In this example, cells are stained with same-clone fluorescent and Ab-oligo antibodies, a 5-plex fluorescent antibody panel and a 10-plex BD® AbSeq Antibody panel.

Note: The antibody pool should be made with 30% overage to ensure the correct amount is added for staining.

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