Single cell RNA-seq library preparation can be an expensive and time-consuming process. Using the BD™ Single-Cell Multiplexing Kits, one can add more samples from a study on a single run for a more controlled experiment.

As a leader in antibody and single cell analysis, BD has developed a method by which users can multiplex samples on a single run of the BD Rhapsody™ Single-Cell Analysis system. By conjugating a polyadenylated DNA barcode to a universal antibody, a single cell sequencing workflow can be multiplexed with up to 12 human samples to reduce reagent cost (Figure 1). Addition of the antibody tags does not affect sequencing data and improves data quality by reducing noise. The BD Single-Cell Multiplexing Kits identifies inter-sample multiplets in downstream data analysis, which are unwanted artifacts during single cell capture. By identifying and eliminating these artifacts in the data analysis, researchers can expect much cleaner and more defined cell populations.

**Product features**

- **Reduces cost:** Reduce single cell library preparation reagent cost by adding more samples to each run
- **Better data:** Lower technical errors between samples for a controlled experiment
- **Decreases noise:** Identify multiplets that have >1 associated Sample Tag
- **Easy workflow:** Simple upstream antibody-labelling step
- **Validated on a BD system:** Compatible with the BD Rhapsody Single-Cell Analysis System
- **Two species supported:** BD™ Human Single-Cell Multiplexing Kit for most human cell types tested and BD™ Mouse Immune Single-Cell Multiplexing Kit with specificity to CD45

<table>
<thead>
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<th>No. of Samples</th>
<th>Standard BD Rhapsody Run</th>
<th>Using the BD Sample Multiplexing Kit on BD Rhapsody Run</th>
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*Does not include the price of BD Sample Tags
Number of loaded cells per sample will vary and decrease for larger sample numbers
Multiplet identification for cleaner single cell sequencing analysis

To determine the viability of using Sample Tags for multiplet identification, ~20,000 cells of 4 different cell types—peripheral blood mononuclear cells (PBMCs), T47D breast cancer cells, Jurkat T cells and Ramos B cells with 3 Sample Tags (ST) each—were loaded in a single BD Rhapsody Cartridge (Figure 2). Sequencing data from BD analysis software revealed 4.9% of the cells were inter-sample multiplets, many of which were located in unique multiplet clusters. Removal of multiplets allows for more representative data analysis of multiple biological samples (Figure 3).

**Figure 2.** Demonstration of multiplet and sample identification.

**Figure 3.** t-SNE analysis of single cell gene-expression profile with the BD Rhapsody™ Immune Response Panel HS. (A) Cells are colored by annotation from the Sample Tag determination algorithm within the BD Rhapsody bioinformatics pipeline. (B) Blue cells highlight multiplets identified. (C) t-SNE plot with multiplets and undetermined cells removed to obtain cleaner data for analysis.

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**BD Single-Cell Multiplexing Kits**

- **633781** BD™ Human Single-Cell Multiplexing Kit
- **633793** BD™ Mouse Immune Single-Cell Multiplexing Kit

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