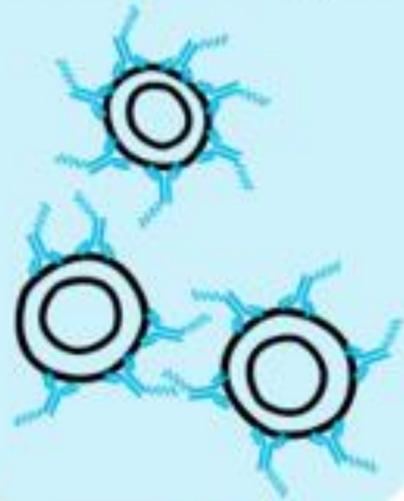


# BD<sup>®</sup> Flex Single-Cell Multiplexing Kit (BD<sup>®</sup> Flex SMK)

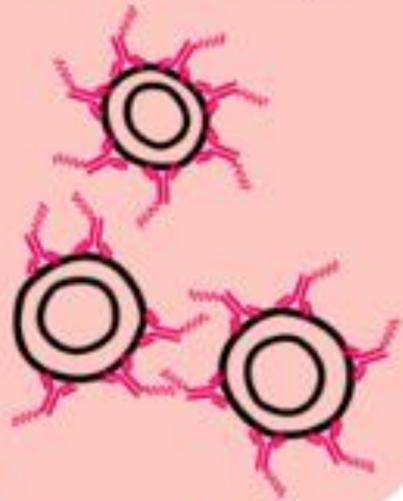
A flexible solution for single-cell sample multiplexing on the  
BD Rhapsody™ Single-Cell Analysis System



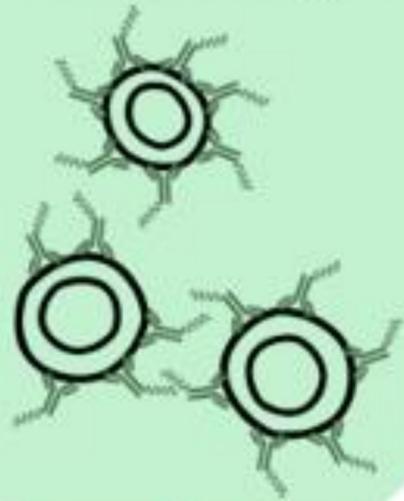
Sample Tag 1



Sample Tag 2



Sample Tag 3



Pool multiple samples into a single lane



Sample Tag demultiplexing by informatics

## BD<sup>®</sup> Single-Cell Multiplexing Kits (BD<sup>®</sup> Flex SMK)

BD<sup>®</sup> Single-Cell Multiplexing Kits utilize an innovative antibody-oligo technology

- To provide high sample throughput for single-cell library preparation
- To improve detection and elimination of multiplet data from single-cell experiments

# Benefits of using BD<sup>®</sup> SMK products with other BD Rhapsody<sup>™</sup> Assays

## Cleaner data with less noise

Identify and remove inter-sample multiplets that have >1 associated Sample Tag in downstream bioinformatics analysis

## Higher single-cell sample throughput

Process and sequence multiple samples in each run for a more controlled experiment

## Significant cost savings

Reduce single cell capture and library preparation reagent cost by multiplexing samples in each run





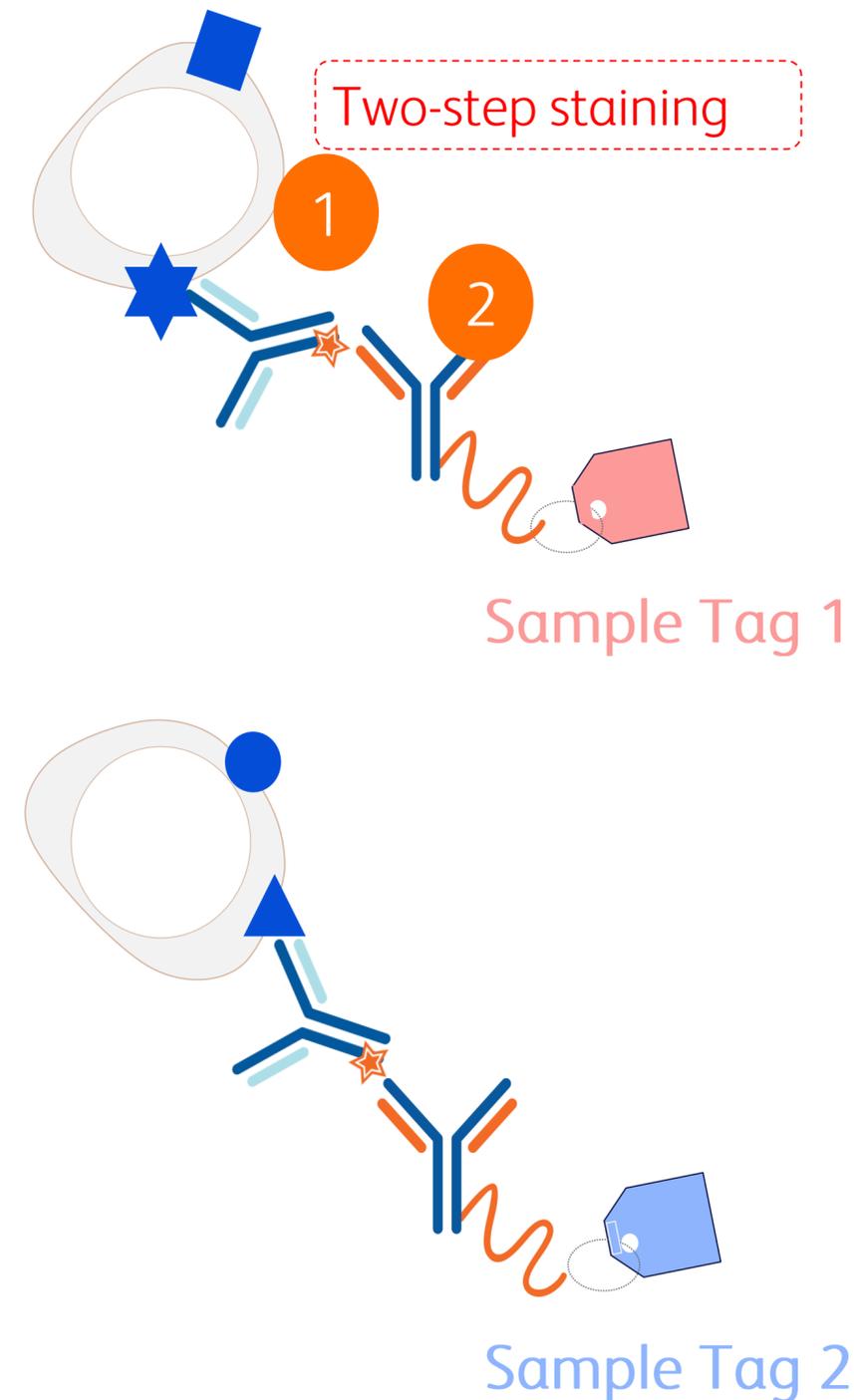
BD<sup>®</sup> Flex Single-Cell Multiplexing  
Kits take single-cell sample  
multiplexing to the next era



BD<sup>®</sup> FLEX SINGLE-CELL MULTIPLEXING KIT  
(BD<sup>®</sup> FLEX SMK)

# BD<sup>®</sup> Flex SMK uses two-step labeling with anti-PE Sample Tags

Every antibody-oligo in the BD<sup>®</sup> Flex Single-Cell Multiplexing Kits has a unique sample oligo barcode conjugated to an anti-R-phycoerythrin (PE), clone E31-1459 antibody. This approach allows you to select any PE-conjugated primary antibody to tag your samples of interest, regardless of species or cell type.



# Now with BD<sup>®</sup> Flex SMK

Further reducing experimental cost

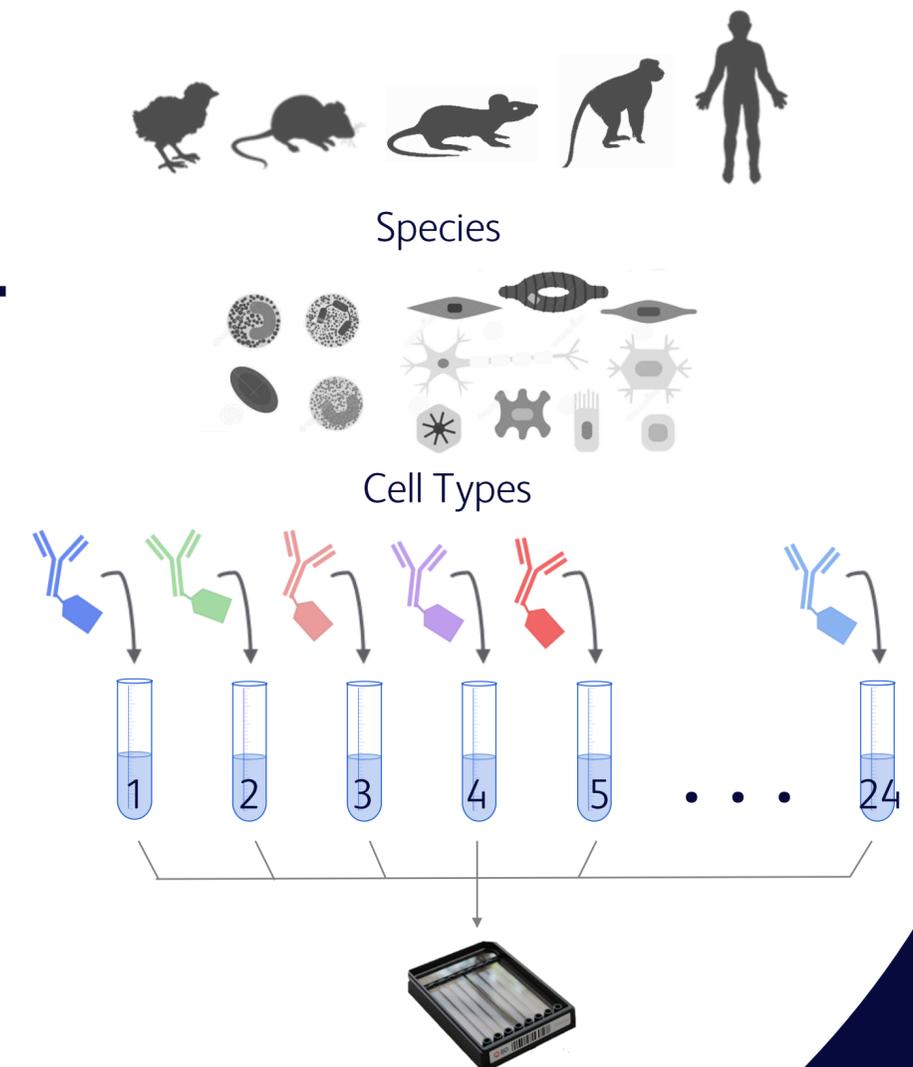
- 6 sample tags per kit with 2 tests per sample tag
- Lower cost/test

For diverse species and cell types

- Enables sample multiplexing for **non-human or -mouse study systems**
- More targeted labeling with **sample-specific primary PE-conjugated antibody**

Supporting higher throughput

- One tube of sample tag labels **2 million cells**
- Up to **24 samples** can be multiplexed by combined use of BD<sup>®</sup> Flex SMK
- Capture more than **40,000 cells** with **~10% multiplet rate**



# An example – BD<sup>®</sup> Flex SMK saves significant reagent cost per sample

If you have 24 samples:

	No sample tag, 1 sample/lane	Multiplexing 12 samples/lane	Multiplexing 24 samples/lane
Lanes required	24	2	1
8-lane cartridge	3 cartridges (6,000)	¼ cartridge (500)	⅛ cartridge (250)
WTA amp kit	6 kits (6,390)	½ kit (532.5)	¼ kit (266.25)
Enhanced cartridge reagent kit	6 kits (19,260)	½ kit (1,605)	¼ kit (802.5)
cDNA kit	6 kits (9,300)	½ kit (775)	¼ kit (387.5)
SMK reagents	-	2 kits (2,680)	4 kits (1 out of 2 tests, 1,500)
Additional antibody	-	-	\$62/25 tests (anti-human CD45 as an example)
Cost per sample (\$)*	1706.25	253.85	133.6

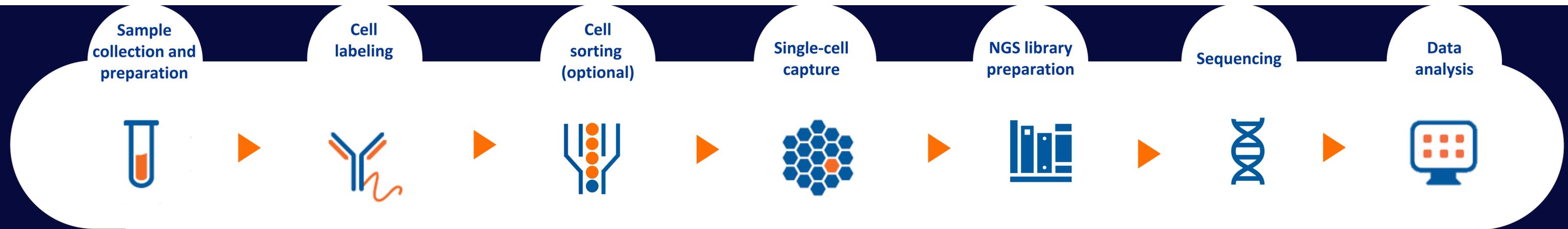
\*Does not include the price of PE-antibodies

\*Number of loaded cells per sample will vary and decrease for larger sample numbers

\*Reagent cost based on U.S. list price as of February 28, 2023



# Single-cell multiplexing workflow on the BD Rhapsody™ System



<p>BD Vacutainer® Tubes</p> <p>or</p> <p>BD Horizon™ Dri Tumor and Tissue Dissociation Reagent (TTDR)</p>	<p>BD® AbSeq Assay (optional) +</p> <p><b>BD® Flex SMK</b></p> <p>+</p> <p>Fluorescent sorting panel (optional)</p>	<p>BD FACS™ Sorters (optional)</p>	<p>BD Rhapsody™ HT Single-Cell Analysis System</p>	<p>mRNA library +</p> <p>BD® AbSeq Assay library (optional) +</p> <p><b>SMK library</b></p>	<p>Illumina™ or</p> <p>Element Biosciences™ Sequencers</p>	<p><b>BD Rhapsody™ Analysis Pipeline</b></p>
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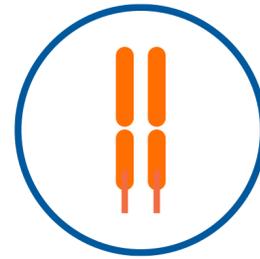
A continuous ecosystem for high-dimensional biology

# BD® Flex SMK is compatible with BD Rhapsody™ Assays



## BD Rhapsody™ Gene Expression Analysis Assays

- Whole Transcriptome Analysis (WTA)
- Targeted mRNA Analysis



## BD Rhapsody™ Immune Repertoire Profiling Assays

- TCR/BCR Multiomic Assay
- VDJ CDR3 Assay



## BD® AbSeq Protein Profiling Assay

- BD® AbSeq Antibody-Oligonucleotides

# Key applications

## Combine >20 samples

Multiplex up to 24 samples and run more than 40K cells in one lane

## Rare cells

Stain up to 2 million cells\* with each Flex SMK Sample Tag and optional BD<sup>®</sup> AbSeq Assay, fluorescent sorting panel, then sort based on primary PE-antibody used for BD<sup>®</sup> Flex SMK\*\*

## Non-human or -mouse samples

Multiplex single-cell samples from rat, monkey, rabbit or other species of interest in your study

## Antigen-specific T cells or B cells

Multiplexing T cell or B cell samples enriched using PE-dextramers or PE-tetramers



# Assay performance

Two datasets showing assay compatibility, BD<sup>®</sup> Flex SMK specifications and comparison between one-step labeling versus two-step labeling



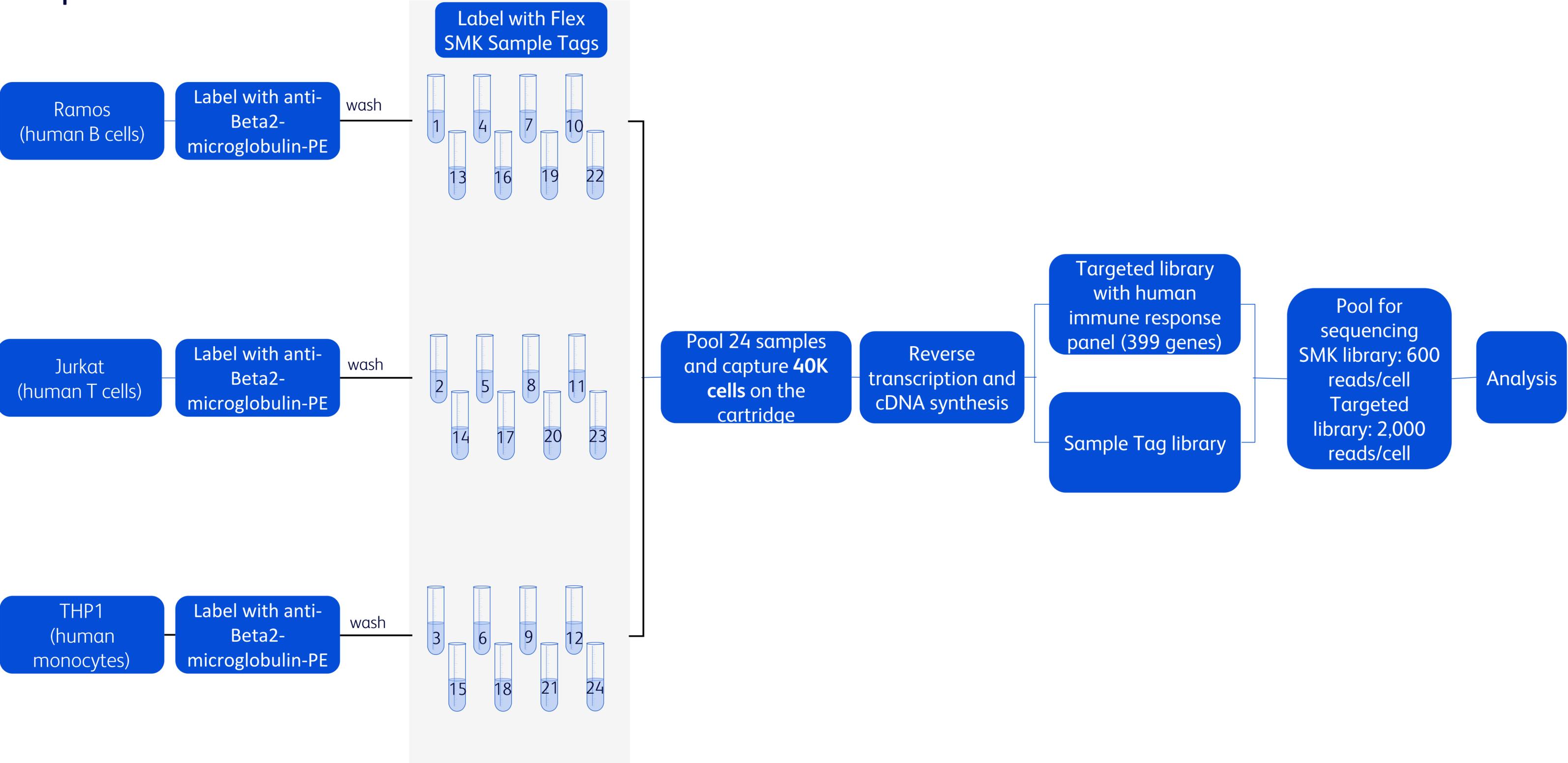
# Dataset 1

Multiplexing 24 samples in a BD Rhapsody™  
Targeted mRNA Assay with BD® Flex SMK

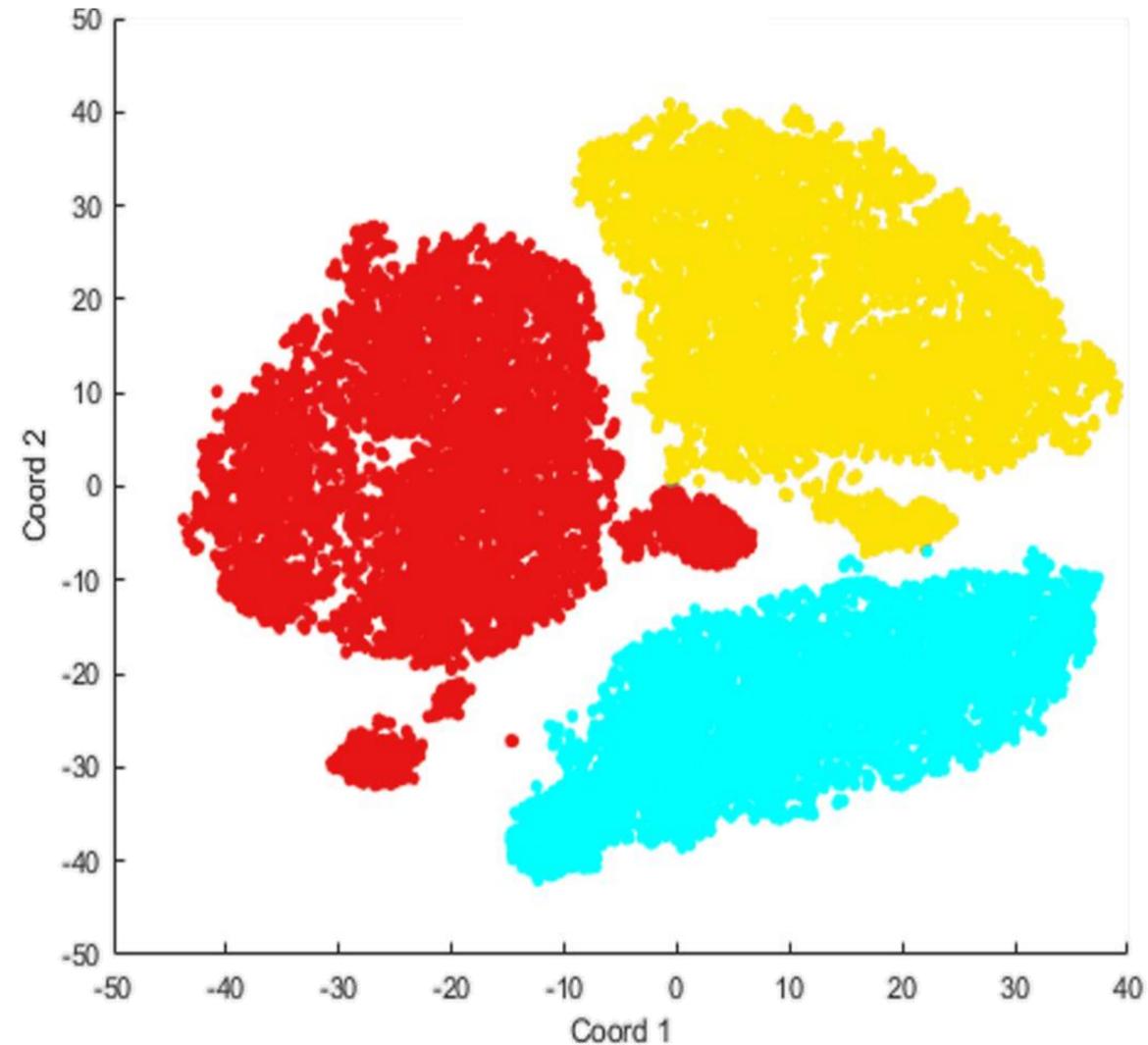


BD® FLEX SINGLE-CELL MULTIPLEXING KIT  
(BD® FLEX SMK)

# Experimental Overview



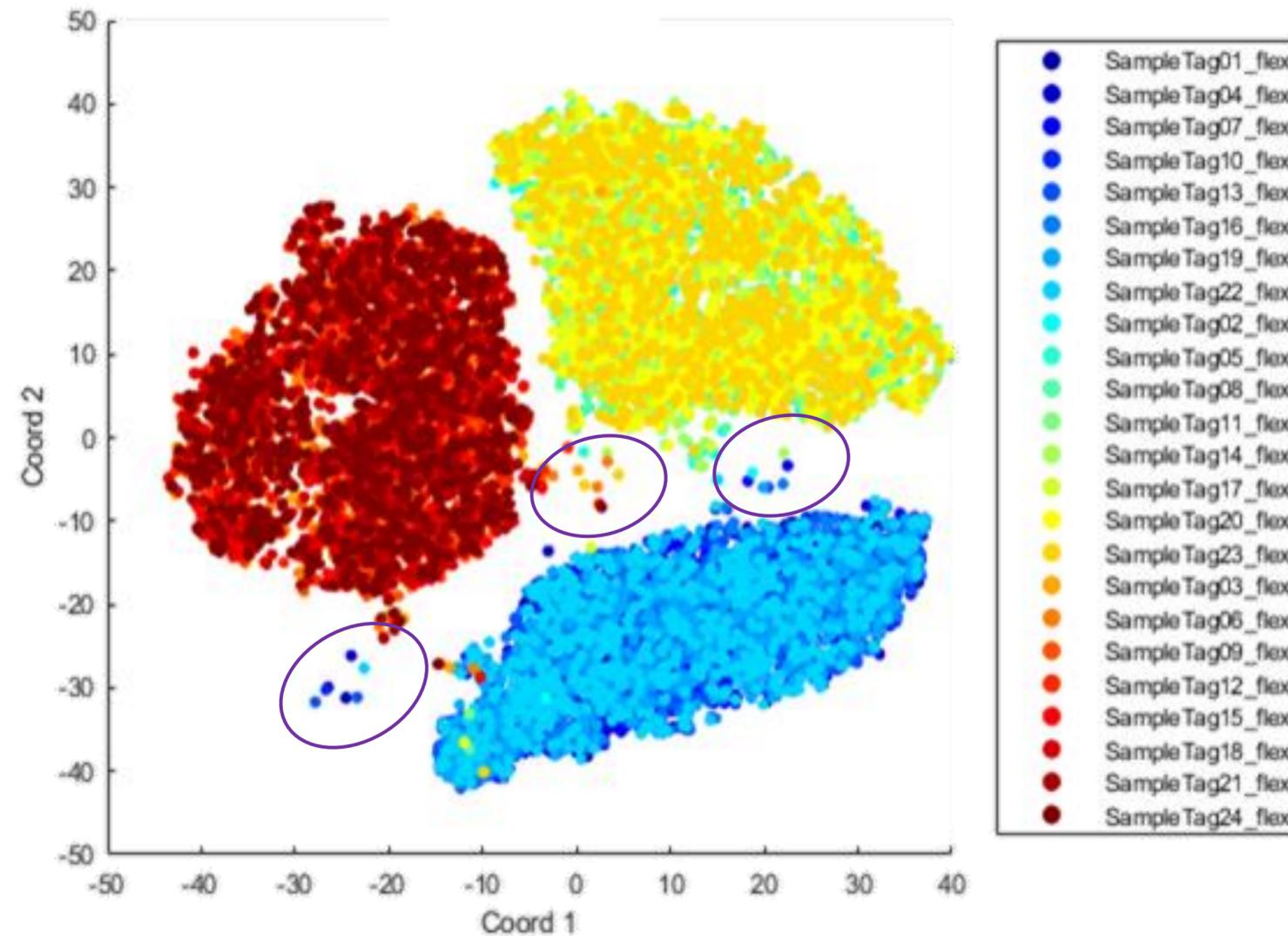
# BD<sup>®</sup> Flex SMK does not impact cell identification and clustering using the BD Rhapsody<sup>™</sup> Targeted mRNA Assay



Cell clusters were generated based on the 399 genes in the Human Immune Response Panel.

The expected three phenotypically distinct cell populations were obtained, corresponding to Human B cell (Ramos), T cell (Jurkat) and Monocytes (THP1)

# 24 Flex SMK Sample Tags labeling shows high SMK sensitivity and specificity



SMK Sensitivity=99.57%

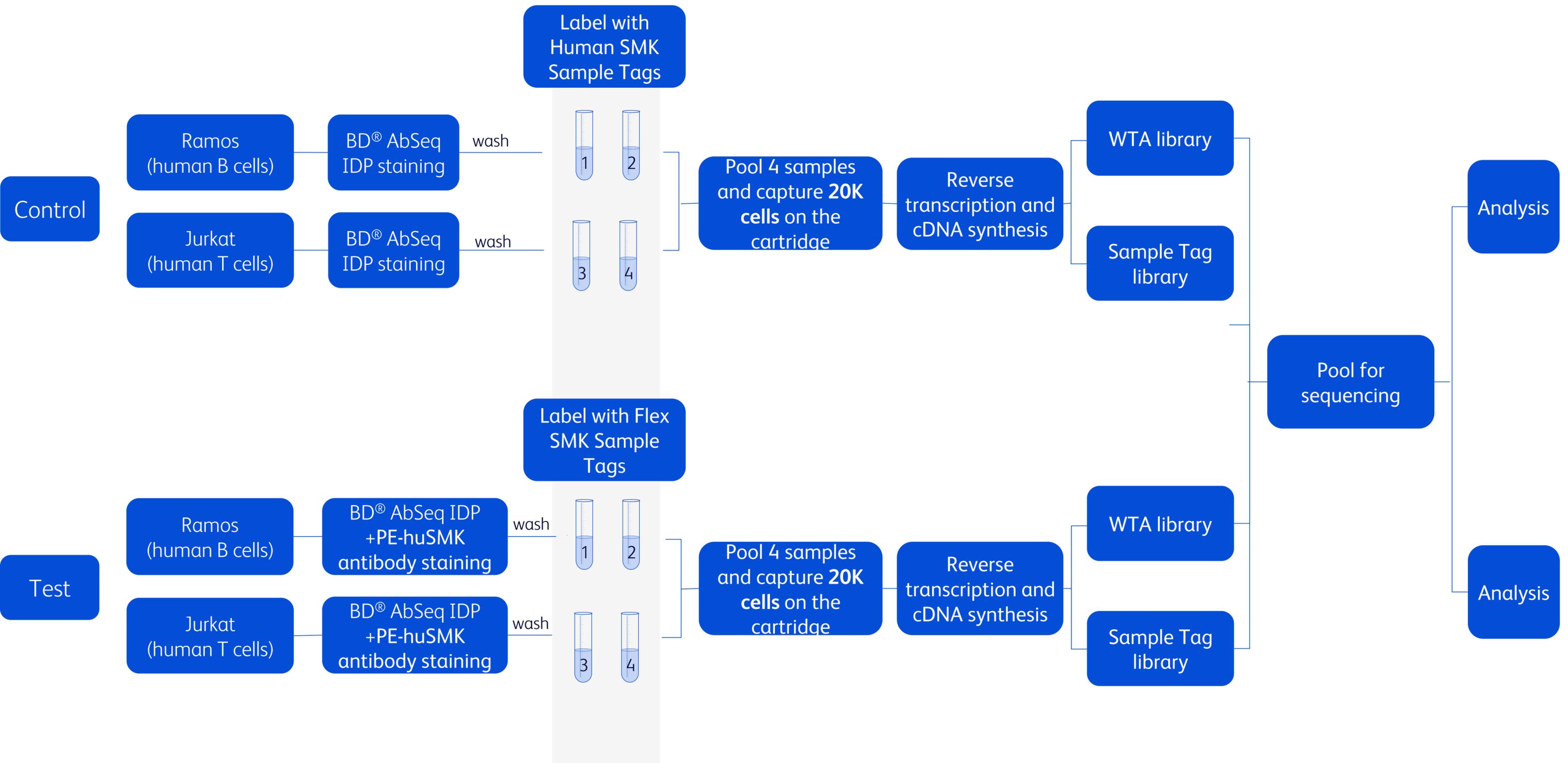
SMK Specificity: All sample tags showed specificity > 95%

Note: Majority of multi-cell populations identified and removed by SMK

# Dataset 2

A comparison between two-step staining versus one-step staining using the BD<sup>®</sup> Flex SMK and BD<sup>®</sup> Human SMK with WTA + AbSeq

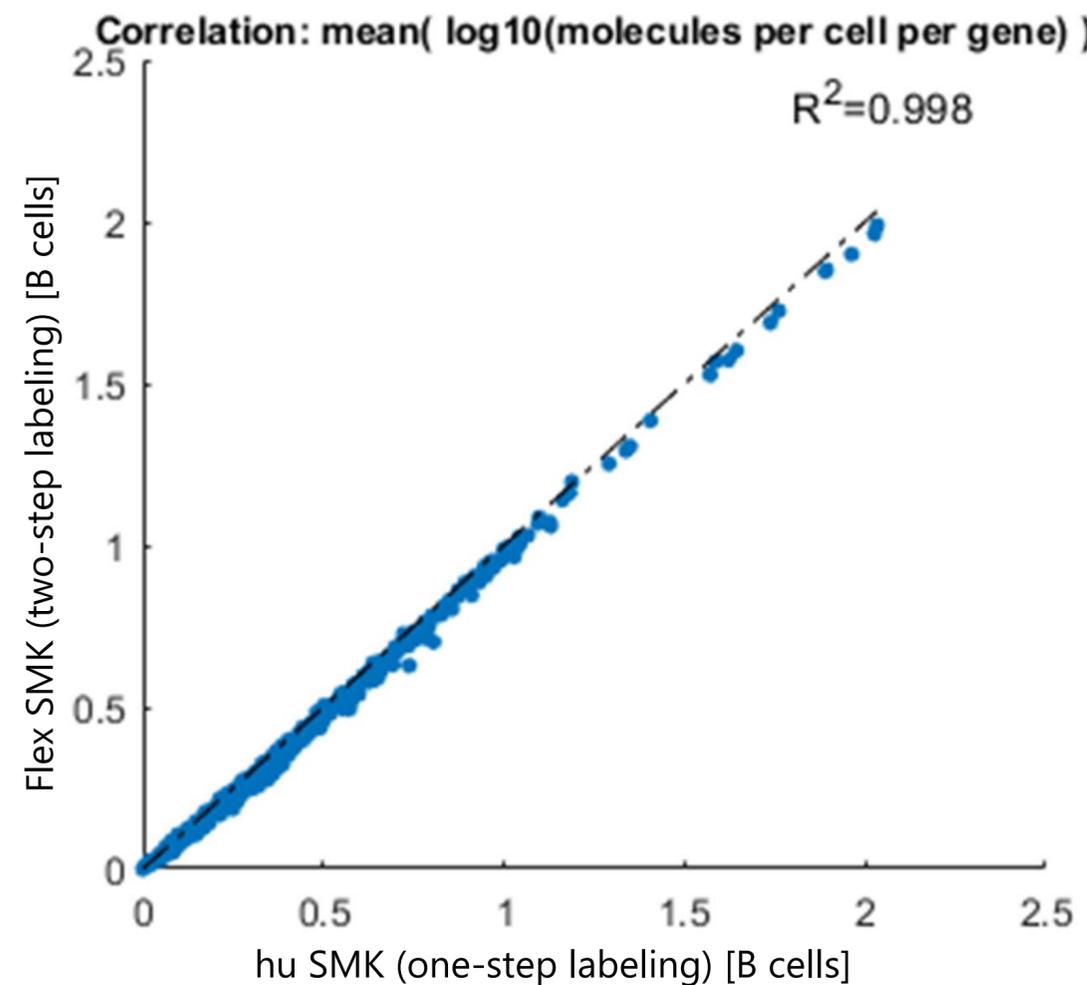
# Experimental overview



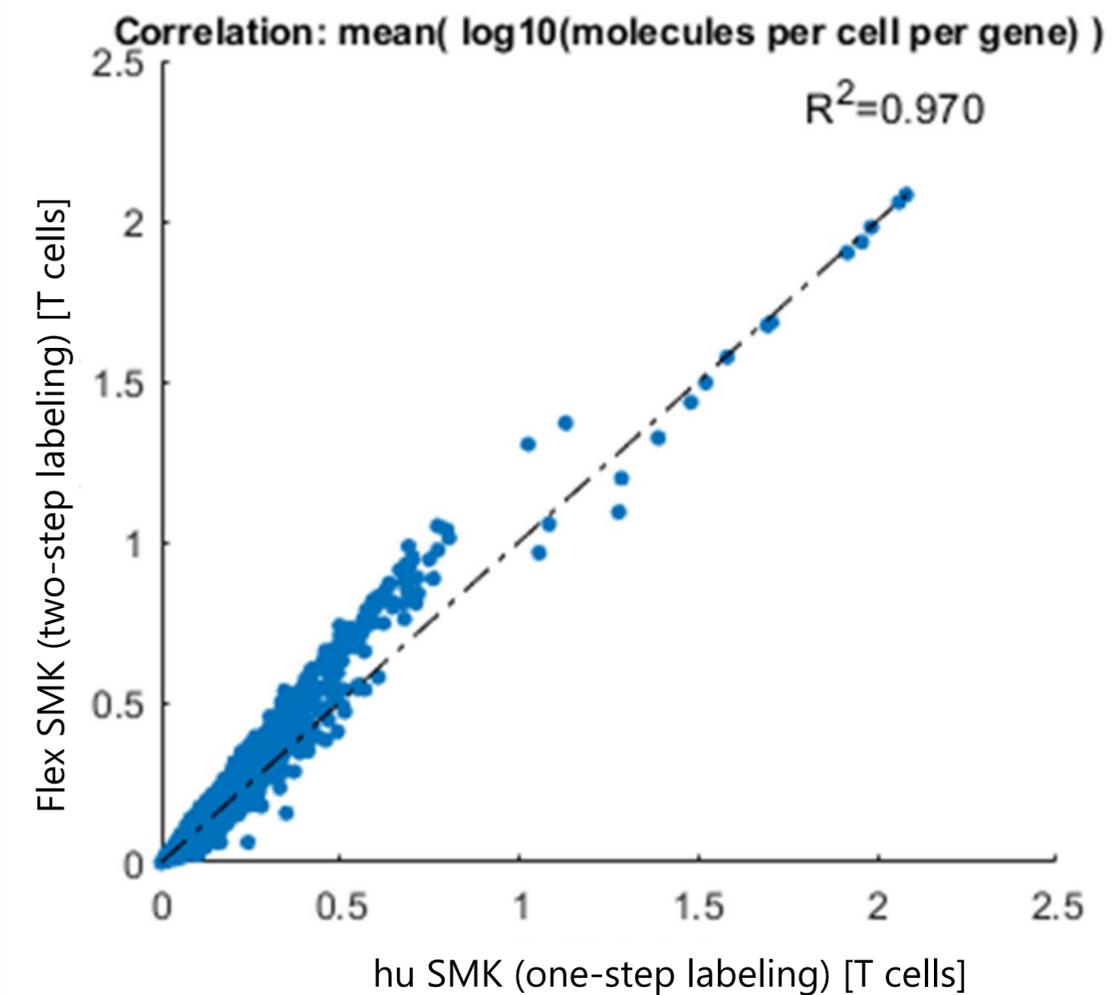
# High WTA mRNA correlation between one-step versus two-step labeling

For both cell types, high mRNA correlations ( $R^2 > 97\%$ ) were obtained between the two workflows, showing similar mRNA recovery from both one-step labeling and two-step labeling workflows

B Cells WTA mRNA Correlation

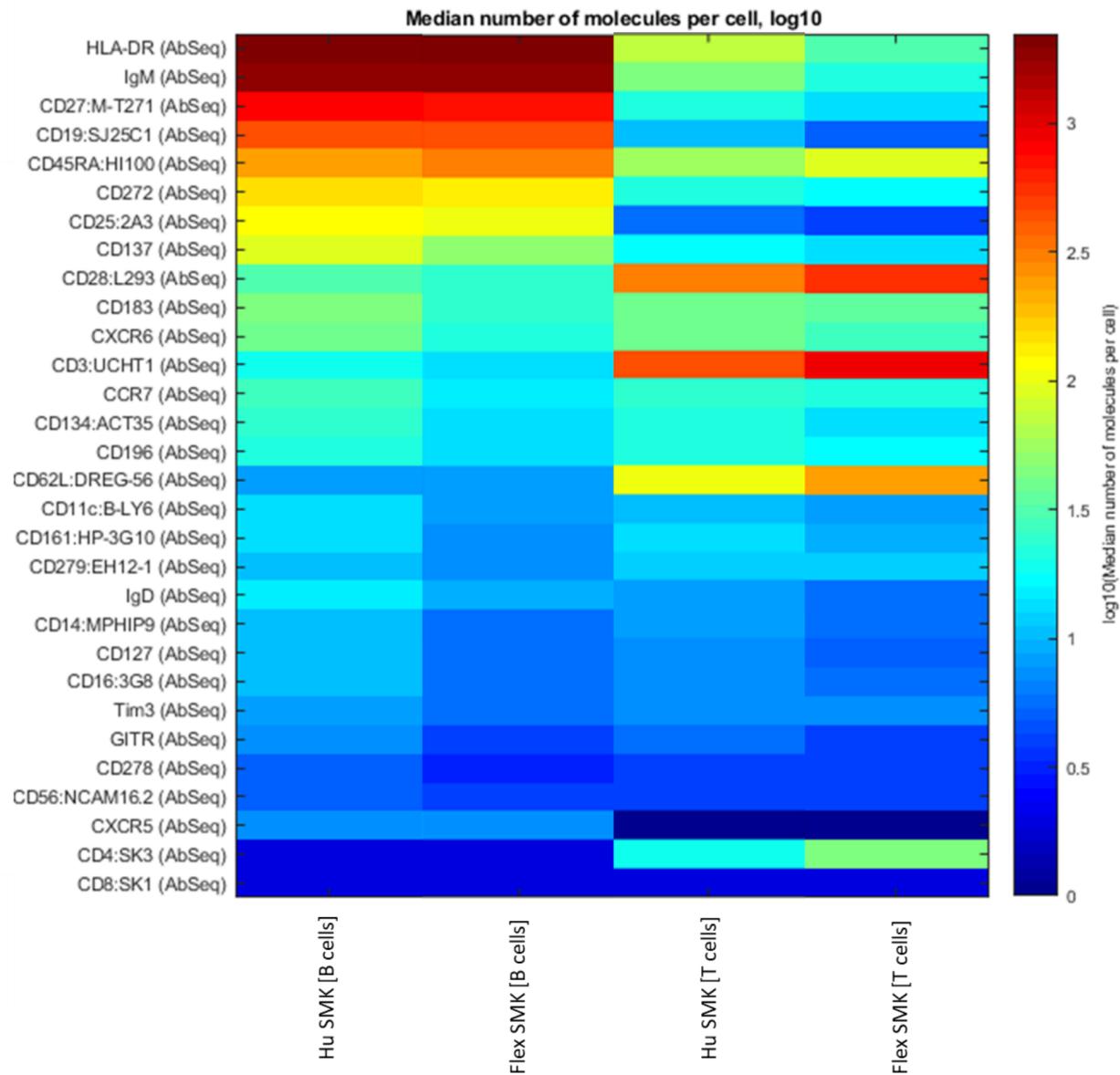
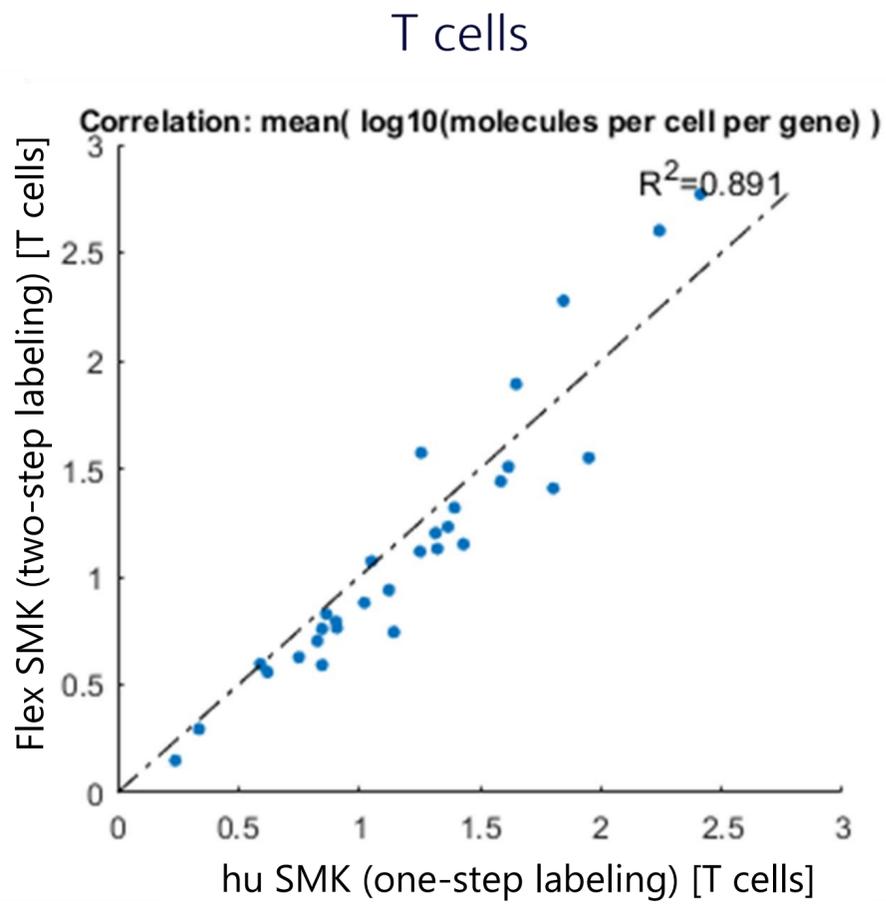
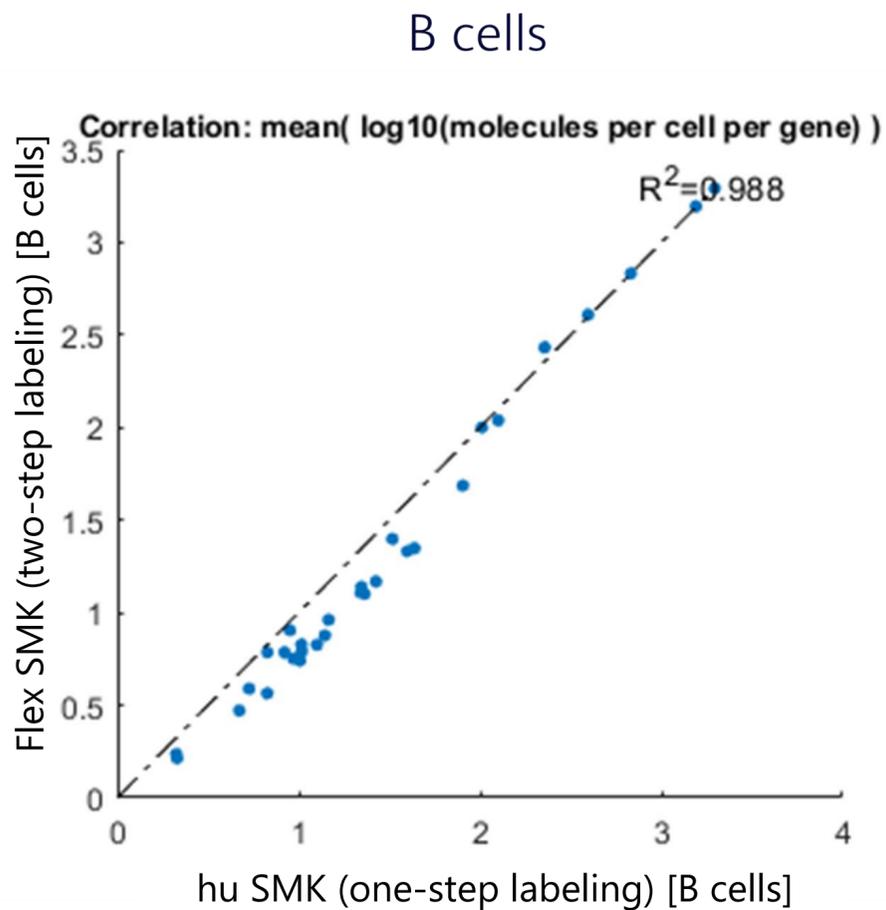


T Cells WTA mRNA Correlation



# Comparable surface AbSeq signal between one-step versus two-step labeling

30-plex surface BD<sup>®</sup> AbSeq Immune Discovery Panel (IDP) was used to co-stain with Flex SMK Sample Tags or Human SMK Sample Tags. The BD<sup>®</sup> AbSeq Assay sensitivity was compared at the individual marker level. High correlation of AbSeq expression between the BD<sup>®</sup> Flex SMK and BD<sup>®</sup> human SMK workflow was obtained.



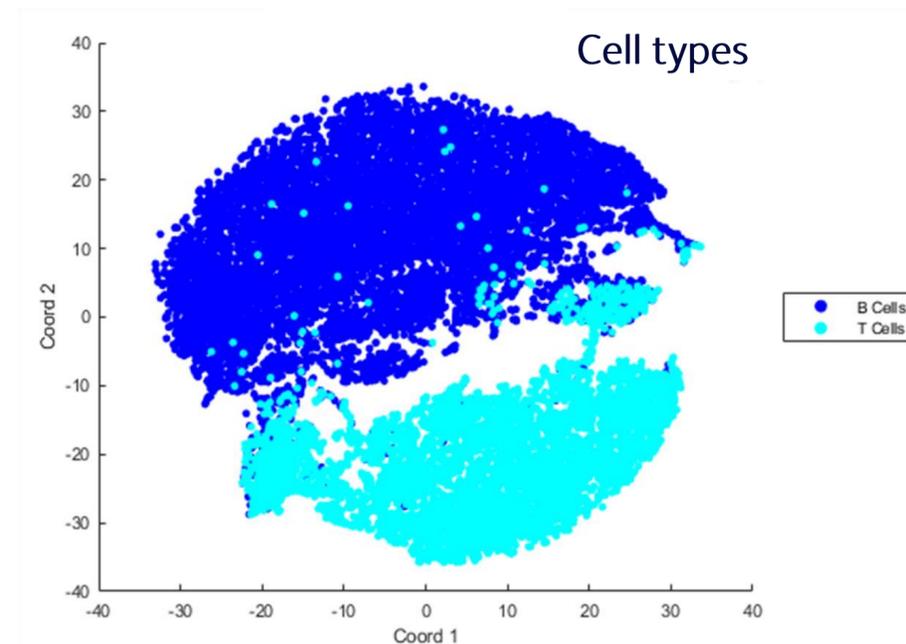
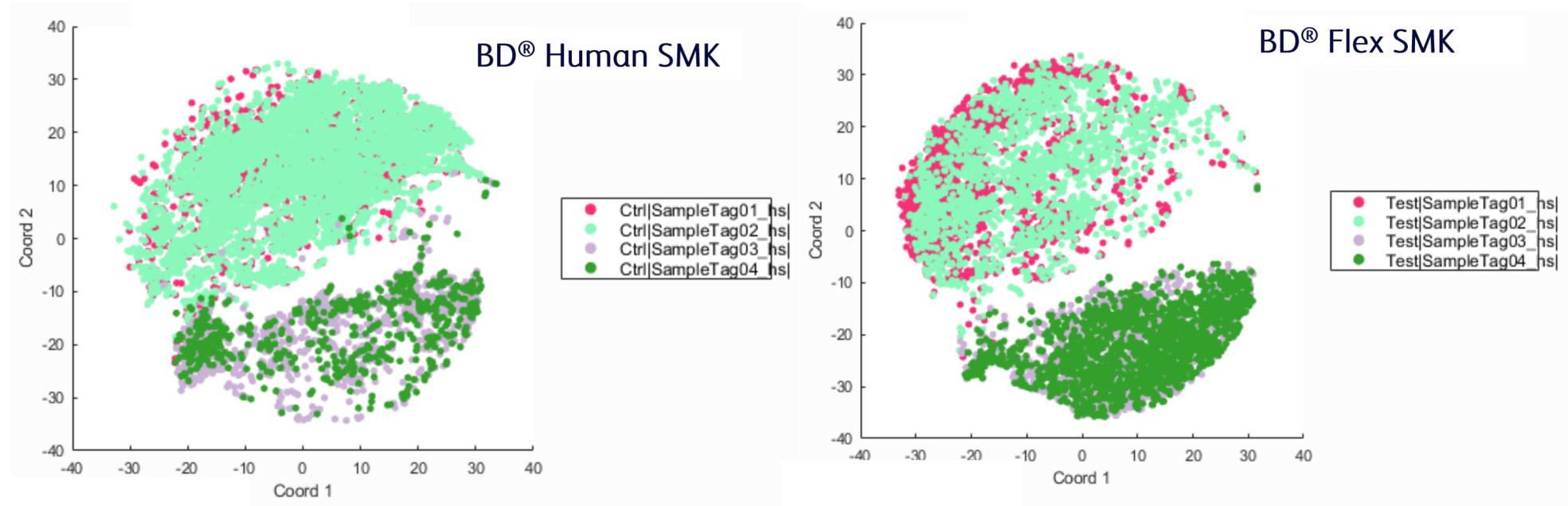
# One-step and two step labeling both show high SMK sensitivity and specificity

## SMK Sensitivity:

- BD<sup>®</sup> Human SMK: 98.9%
- BD<sup>®</sup> Flex SMK: 99.16%

## SMK Specificity:

All human and Flex SMK Sample Tags showed >94% specificity to expected cell types.



# Conclusions

Up to 24 samples can be multiplexed in one single-cell multiomics experiment using BD<sup>®</sup> Flex SMK

BD<sup>®</sup> Flex SMK shows high SMK sensitivity and specificity

BD<sup>®</sup> Flex SMK is compatible with BD Rhapsody<sup>™</sup> Multiomic Assays

The two-step labeling workflow of BD<sup>®</sup> Flex SMK does not impact SMK assay performance

# Experimental considerations, sequencing recommendations and analysis pipeline setup



# Experimental considerations

## Required material

One test of BD<sup>®</sup> Flex SMK Sample Tag can stain **50,000–1 million cells**. We recommend pooling more cells (up to one million cells) than you want captured in the BD Rhapsody<sup>™</sup> Cartridge, because there can be cell loss during BD<sup>®</sup> Flex SMK labeling and washing.

## Selection of primary antibody

Selection of primary PE-conjugated antibody and staining concentration can impact the performance of BD<sup>®</sup> Flex SMK. Ideally the target(s) will be **uniformly expressed at high levels on all the cell types in your sample**. Expression level and optimal titration of antibody on the sample of interest can be predetermined by flow cytometry.

## Use with other BD<sup>®</sup> SMK products

Flex SMK Sample Tag barcode sequences are the same as Human and Mouse Sample Tags. Refer to Appendix in the BD<sup>®</sup> Flex SMK staining protocols\* for instructions on combined use with other BD<sup>®</sup> SMK products



# Sequencing recommendations

## Pooling samples of the same type

- Recommendation: 120 reads/cell
- for example, combining different donor PBMCs.

## Pooling different sample types

- Recommendation: 600 reads/cell
- for example, combining Jurkat cells with PBMCs.

▼ Multiplexing\_Settings (#Multiplexing\_Settings)

▼ Sample Tag Names ? ✎ +

 This input is set to null.

Sample Tags Version ?

No value ▼

No value

▼ No Multiplexing

Single-Cell Multiplex Kit - Human

Single-Cell Multiplex Kit - Mouse

Single-Cell Multiplex Kit - Flex

## BD Rhapsody™ Analysis Pipeline multiplexing settings

- Create task using the Targeted or WTA pipeline (v1.12 or later versions)
- In Multiplexing\_Settings, select **Single-Cell Multiplexing Kit - Flex** under Sample Tags Version

# Ordering information and available protocols



BD® FLEX SINGLE-CELL MULTIPLEXING KIT  
(BD® FLEX SMK)

# BD<sup>®</sup> Flex Single-Cell Multiplexing Kits



Four BD<sup>®</sup> Flex Single-Cell Multiplexing Kits are available, each consisting of six Sample Tags with unique sample oligo barcodes. These four kits are fully compatible, allowing up to 24 samples to be labeled and pooled prior to single-cell capture with the BD Rhapsody<sup>™</sup> System.

Catalog Number	Product
633849	BD <sup>®</sup> Flex Single-Cell Multiplexing Kit A
633850	BD <sup>®</sup> Flex Single-Cell Multiplexing Kit B
633851	BD <sup>®</sup> Flex Single-Cell Multiplexing Kit C
633852	BD <sup>®</sup> Flex Single-Cell Multiplexing Kit D

 **BD Rhapsody™ System**  
Single-Cell Labeling with  
BD® Flex Single-Cell Multiplexing Kits  
Protocol

 **BD Rhapsody™ System**  
Single-Cell Labeling with  
BD® Flex Single-Cell Multiplexing Kits and  
BD® AbSeq Ab-Oligos  
Protocol

23-24311(01)  
2022-12

For Research Use Only

23-24312(01)  
2022-12

For Research Use Only

## BD® Flex SMK cell staining protocols

- BD Rhapsody™ System Single-Cell Labeling with BD® Flex Single-Cell Multiplexing Kits Protocol (Doc ID: 23-24311)
- BD Rhapsody™ System Single-Cell Labeling with BD® Flex Single-Cell Multiplexing Kits and BD® AbSeq Ab-Oligos Protocol (Doc ID: 23-24312)

# Support

Utilize our expertise and insights for your single-cell experiments.

Reach out to your local BD sales representative or contact our help desk [scomix@bdscomix.bd.com](mailto:scomix@bdscomix.bd.com) to learn more about using the BD<sup>®</sup> Flex SMK or other BD<sup>®</sup> SMK products



# Appendix



BD<sup>®</sup> FLEX SINGLE-CELL MULTIPLEXING KIT  
(BD<sup>®</sup> FLEX SMK)

# BD<sup>®</sup> SMK product information

Catalog number	Product
633781	BD <sup>®</sup> Human Single-Cell Multiplexing Kit
633793	BD <sup>®</sup> Mouse Immune Single-Cell Multiplexing Kit
626545	Custom BD <sup>™</sup> Single-Cell Multiplexing Set Rat Anti-Mouse MHC-H2 Class I (M1/42)



# Thank you



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