BioLegend®

Multiomics With TotalSeq™ Reagents

Uncover What Makes Each Cell Unique

World-Class Quality | Superior Customer Support | Outstanding Value

Multiomics has transformed traditional sequencing experiments by providing simultaneous protein and genetic analysis to an unparalleled depth. We offer a comprehensive library of products, including antibodies, panels, and cell hashtags for sample multiplexing, to enable protein detection by sequencing. Explore the capabilities of TotalSeq™ reagents and see how they seamlessly integrate into existing and novel workflows. to uncover what makes each cell unique.

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Single-Cell Protein and RNA Detection

TotalSeq™ oligo-conjugated antibodies enable measurement of proteins at a single-cell level in applications that integrate simultaneous nucleic acid and protein detection, such as CITE-seq or REAP-seq. They integrate seamlessly into existing single-cell RNA sequencing workflows, including capture methods that use poly(dT) – mRNA poly(A) hybridization, as well as those workflows available from 10x Genomics.

Simultaneous Multiomic Data Generation

Increase the power of single-cell experiments by combining proteomic and transcriptomic data.

Reduced dropouts: TotalSeq™-derived antibody tags are not prone to dropouts.

Enhanced cell clustering and identification: Some RNA molecules are expressed at low levels or variants are difficult to detect at the RNA level, such as CD45A and CD45RO. Using TotalSeq™ antibodies overcomes these limitations, providing enhanced cell identification.

Ultra-high parameter protein detection: Detect proteins on a massively parallel scale by multiplexing 100 or more antibodies in a single experiment.

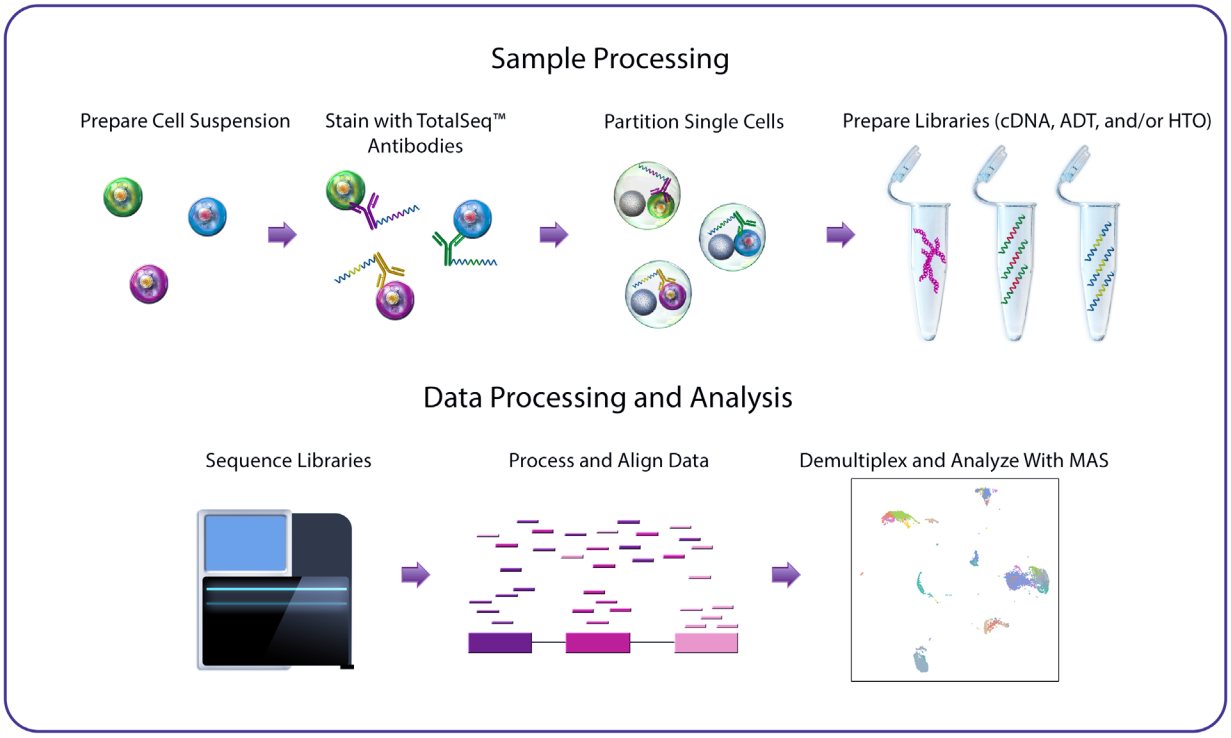
Increased efficiency: Easily combine multiple samples with our cell hashing antibodies to improve workflows and reduce experimental cost.

Diverse applications: With a wide range of mouse and human targets available, you can use TotalSeq™ antibodies in a variety of research areas, including:

- Personalized or Precision Medicine
- Cancer Research
- Stem Cell Research
- Basic and Applied Immunology
- Biomarker Discovery
- Characterization of New or Rare Cell Types
- Neuroimmunology
- Vaccine Research

Learn more: biolegend.com/en-us/totalseq/single-cell-rna

Simultaneous Single-Cell Protein and RNA Detection Workflow



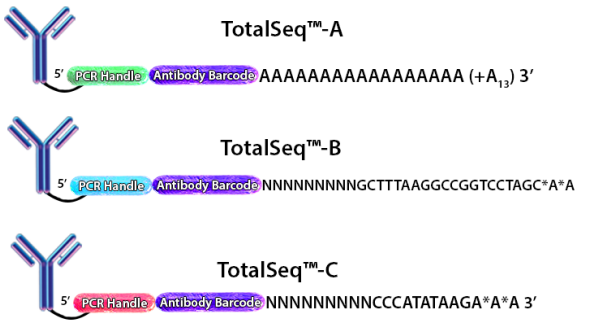
Antibody Formats for Single-Cell Protein and RNA Analysis

Leveraging our portfolio of trusted antibody clones, we offer the largest selection of oligo-barcoded antibodies. Antibodies are available in multiple formats so you can design customized experiments to answer your unique research questions.

Each TotalSeq™ antibody is conjugated to a unique oligonucleotide containing a

1. Capture sequence
2. Clone-specific barcode sequence
3. PCR handle compatible with Illumina® sequencing reagents and instruments.

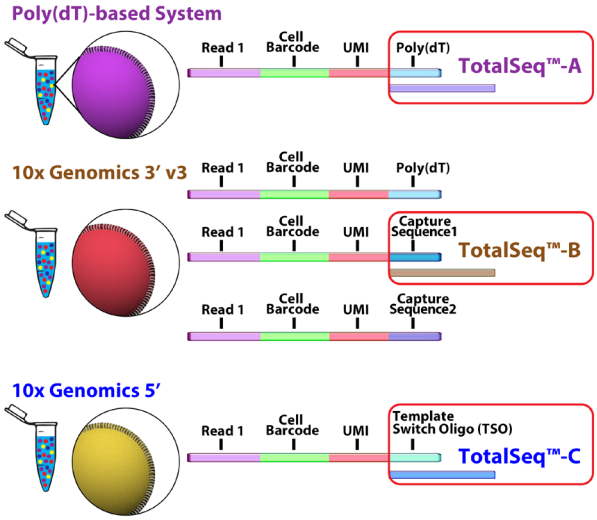
The TotalSeq™ oligonucleotide that is conjugated to our antibodies is also referred to as an antibody-derived tag (ADT) or hashtag oligonucleotide (HTO) depending on the application and use.



TotalSeq™-A: Designed to work with any sequencing platform that relies on poly(dT) oligonucleotides as the mRNA capture method. TotalSeq™-A antibodies contain a poly(A) sequence which mimics a natural mRNA.

TotalSeq™-B: Capture sequence is compatible with 10x Genomics' Chromium Single Cell Expression Solution 3' kit with Feature Barcode Technology (v3 or later).

TotalSeq™-C: Capture sequence is compatible with 10x Genomics' Chromium Single Cell Immune Profiling Solution 5' kit which allows for immune repertoire profiling of T and B cell receptors at a single-cell resolution.



Compare and Contrast Antibody Formats

	TotalSeq™-A	TotalSeq™-B	TotalSeq™-C
10x Genomics Single-Cell Platform Compatibility	Single Cell Gene Expression Solution (3', v2 or later) and any system employing the poly(A) tail capture method	Single Cell Gene Expression Solution (3', v3 or later) with Feature Barcoding Technology and 10x Genomics Data Analysis Software[1]	Single Cell Immune Profiling Solution (5') with Feature Barcoding Technology and 10x Genomics Data Analysis Software[1]
PCR handle	CCTTGGCACCCGAGAATTCCA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNN[2]	CGGAGATGTGTATAAGAGACAGNNNNNNNNNN
Capture sequence	Poly-A [(A)30*A*A[3]]	NNNNNNNNNGCTTTAAGGCCGGTCCTAGC*A*A[4]	NNNNNNNNNCCCATATAAGA*A*A[4]
Next-generation sequencing compatibility	Compatible with Illumina instruments	Compatible with Illumina instruments	Compatible with Illumina instruments

Notes:

1. *10x Genomics Data Analysis Software does not support cell hashing analysis.*
2. *N represents random short nucleotide sequences that prevent amplification biases.*
3. *The symbol * indicates a phosphorothioated bond. This is added to prevent nuclease degradation.*
4. *These sequences are unique to the TotalSeq™-B and -C conjugates, and they were developed independently of the reagents used by researchers at the New York Genome Center (NYGC, CITE-seq.com). TotalSeq™-B, -C, and the antibodies used by NYGC are all compatible with 10x Genomics Solutions, but they utilize different oligo sequences. As such, protocols and additional reagents, including required primers, differ between the antibody formats. Please refer to our protocols when using TotalSeq™ reagents.*

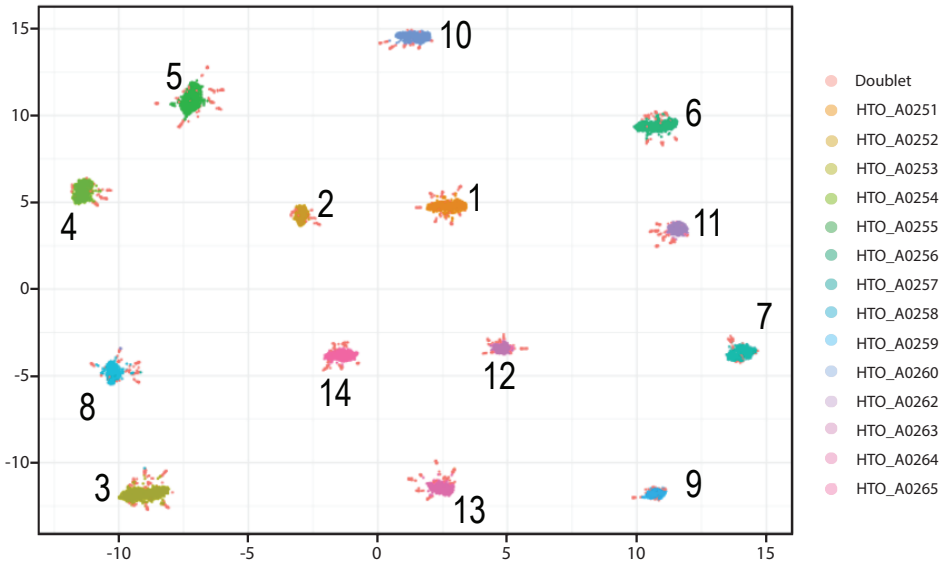
Sample Multiplexing or Hashtags

Cell Hashing

To pool multiple samples prior to loading them onto a platform capable of single-cell isolation, try one of our hashtag reagents. Each ready-to-use hashtag reagent contains a pool of antibodies designed to recognize ubiquitously expressed cell surface markers and is conjugated to a unique barcode. For human samples, our hashtag reagents recognize CD298 and β2-Microglobulin, and for mouse samples, hashtag antibodies recognize CD45 and H-2 MHC Class I.

Benefits of using cell hashing:

- Robust multiplet identification.
- Minimize variability and reduce batch effects between samples.
- Pool smaller samples to reach minimal cell numbers.
- Optimize experimental cost.



Human PBMCs from a single donor were stained separately with 14 TotalSeq™-A Hashtags and pooled into a single sample. The samples were then subject to CITE-seq using 10x Genomics' Chromium Single Cell Expression 3' Kit v3.1. The UMAP coordinates were generated using asinh-transformed UMI count matrix. The same matrix was used to demultiplex with DemuxEM algorithm, and the results were used to color the cell clusters on the UMAP plot.

Hashtag Formats and Considerations

We offer hashtag reagents in our TotalSeq™-A, B, and –C formats. Hashtag reagents from all formats will function similarly, but the workflow and required primers differ.

TotalSeq™-A Hashtag Reagents:

- 14 human and 15 mouse hashing antibodies available.
- Antibody derived tag (ADT) and hashtag oligo (HTO) libraries are independently constructed which can allow for further sequencing optimization.

TotalSeq™-B and –C Hashtag Reagents:

- 10 human and 10 mouse hashing antibodies available in each format.
- Antibody derived tag (ADT) and hashtag oligo (HTO) libraries are constructed together as a single library.
- Hashtag antibodies must be titrated during staining to optimize sequencing depth.

Nuclear Hashing

Single-nucleus RNA-seq makes it possible to characterize cellular states and physiology in tissues that are challenging to dissociate. This includes tissues that are rich in certain cell types like neurons, adipocytes, and muscle cells. Single-nucleus RNA-seq can also help when tissue storage is required, as it is difficult to recover and obtain single-cell suspensions from archived frozen material.

To pool isolated nuclei from different samples, we developed nuclear hashing reagents. Our nuclear hashtag antibodies recognize a family of nuclear pore complex proteins and can cross-react with a wide range of eukaryotic organisms, including human, mouse, and rat cells, as well as other animal models such as Xenopus and yeast.

Antibody Cocktails

Examine over 100 cell surface markers in a single experiment using optimized TotalSeq™ antibody lyophilized cocktails. Each single-use tube contains a pre-titrated amount of each antibody, removing the need for additional optimization and minimizing the variability between experiments.

Benefits of Using TotalSeq™ Cocktails:

- Provided in a convenient, single-use tube.
- Lower cost when compared to buying individual antibodies.
- Pre-titrated antibodies for optimal performance.
- Minimizes variability between different experiments, different labs, and during longitudinal studies.

Universal Cocktails

Take a deeper look at immune cells with our TotalSeq™ Human Universal Cocktails, available in TotalSeq™-A and C formats. Each cocktail contains over 125 antibodies and their associated isotype controls for large-scale protein detection. Within the cocktail, each antibody has been individually titrated using next-generation sequencing as a read-out to provide optimal discrimination of positive and negative cell populations.

TotalSeq™-A Universal Cocktail v1.0

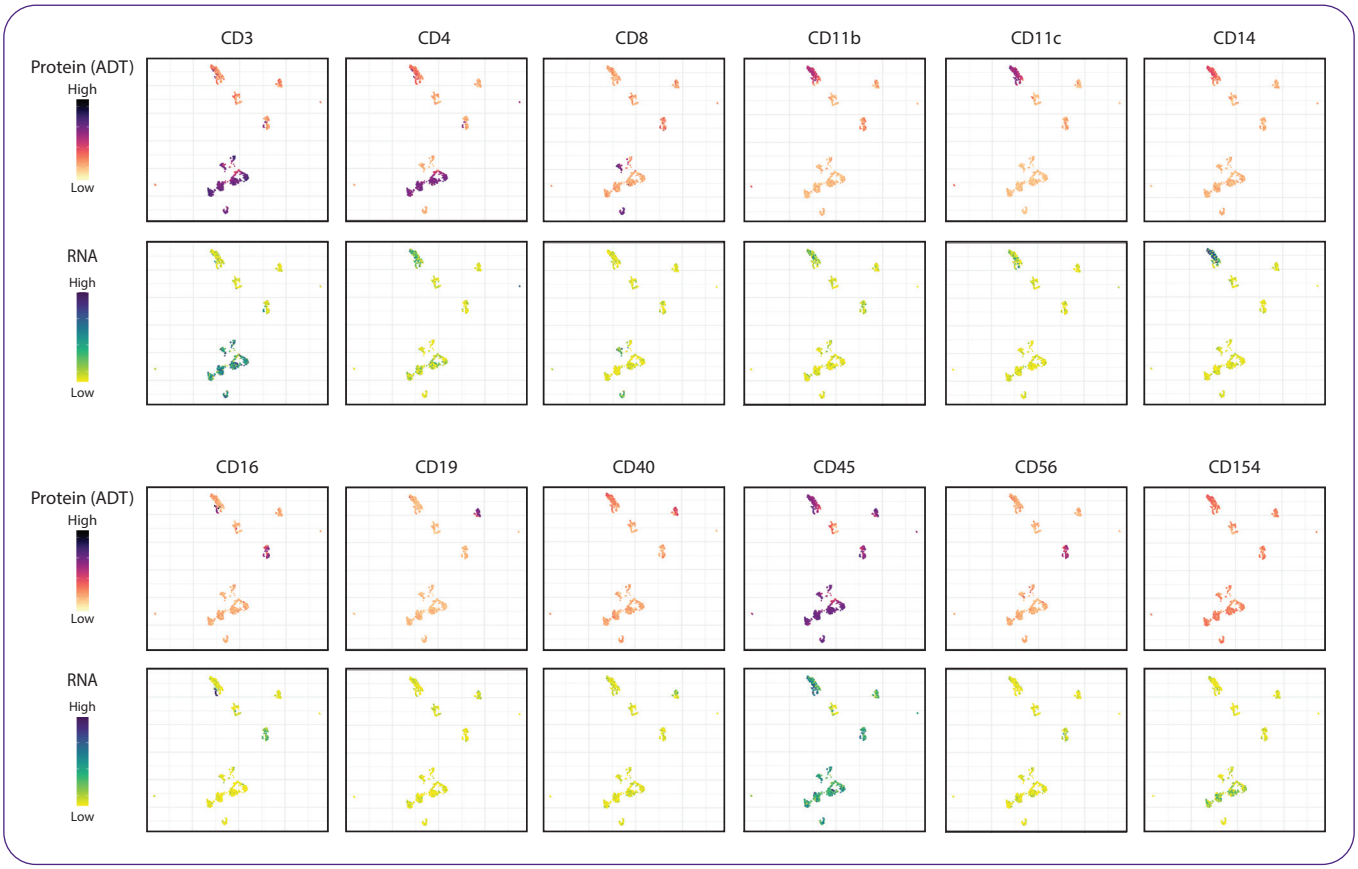
- Compatible with any single-cell platform that uses poly(dT) oligonucleotides as the mRNA capture method.
- Contains 154 primary antibodies and 9 isotype controls.

TotalSeq™-B Universal Cocktail v1.0

- Compatible with 10x Genomics Chromium Single Cell Gene Expression Kits with Feature Barcode technology (v3, v3.1, v3.1 dual index) for cell surface protein.
- Contains 134 primary antibodies and 6 isotype controls.

TotalSeq™-C Universal Cocktail v1.0

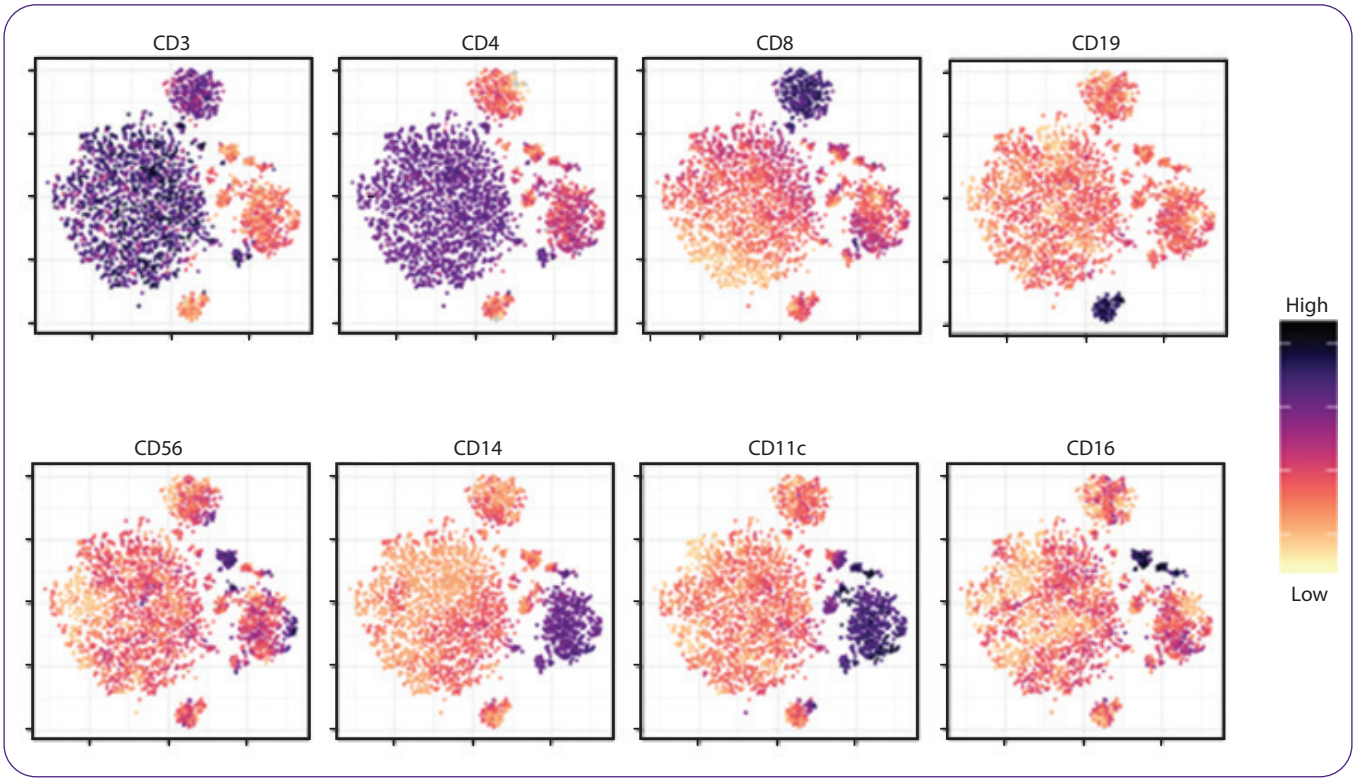
- Compatible with v1 and v2 of 10x Genomics' Single Cell Immune Profiling Solution, allowing you to obtain cell surface protein expression, transcript expression, and full-length paired B and T cell receptor sequences.
- Contains 130 primary antibodies and 7 isotype controls.



Human PBMCs were stained with the TotalSeq™-A Human Universal Cocktail v1.0 and processed using 10x Genomics' Chromium Single Cell Expression 3' Kit v3.1 and Illumina sequencing. Protein and RNA count data were transformed and visualized in a UMAP projection overlaid with protein and RNA expression levels. Clusters were identified based on protein expression only.

TBNK Cocktails

Our TBNK Panels are designed to identify T, B, and NK cells as defined by the expression of CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD45, and CD56. The TBNK cocktail is available in TotalSeq™-A, B, or C formats.



Human PBMCs were stained with the TotalSeq™ Human TBNK panel containing antibodies against CD3, CD4, CD8, CD19, CD56, CD14, CD11c, CD16 and processed using 10x Genomics' Single Cell 3' v3 feature barcoding kit and Illumina sequencing. Protein count data were transformed and visualized in a UMAP projection overlaid with protein expression levels for each component of the cocktail. Clusters were identified based on protein expression only.

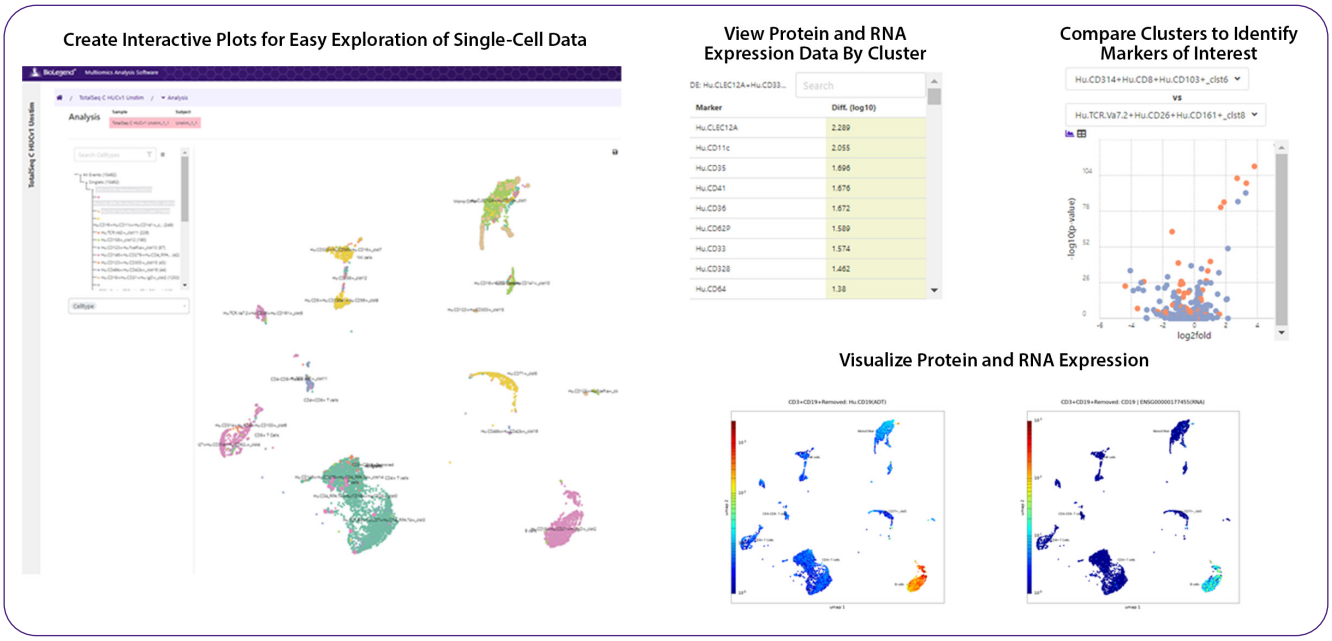
Missing a critical marker for your study? Add single antibodies to any of our panels to expand the panel and include the markers you need. Browse our wide selection of individual antibody conjugates to find more targets.

Multiomics Analysis Software (MAS)

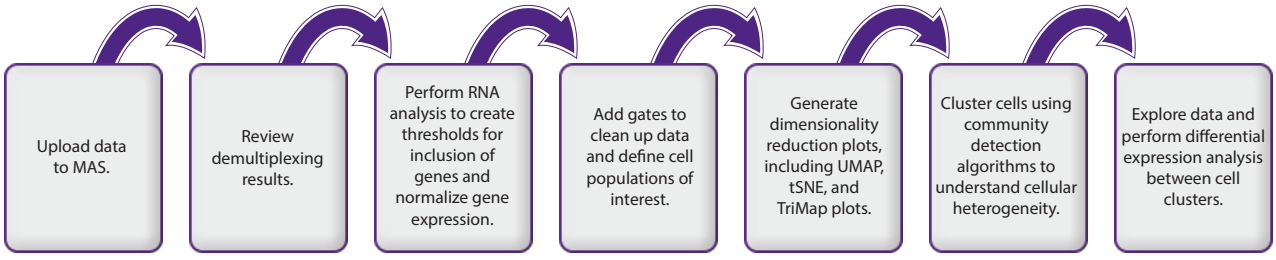
Analyzing single-cell multiomics data often requires advanced tools and extensive expertise. The ability to successfully analyze data can depend on the availability of resources and a researcher's comfort level with the application. To make single-cell data analysis easier, we have developed Multiomics Analysis Software (MAS), a free, cloud-based program to quickly and easily explore CITE-seq data without needing an in-depth bioinformatics background.

Features of the software include:

- Simple, easy-to-use user interface.
- Accessibility through any web browser.
- Flow cytometry-like gating based on TotalSeq™ antibody staining.
- Creates UMAP, tSNE, or TriMAP dimensionality reduction plots.



Recommended MAS Workflow

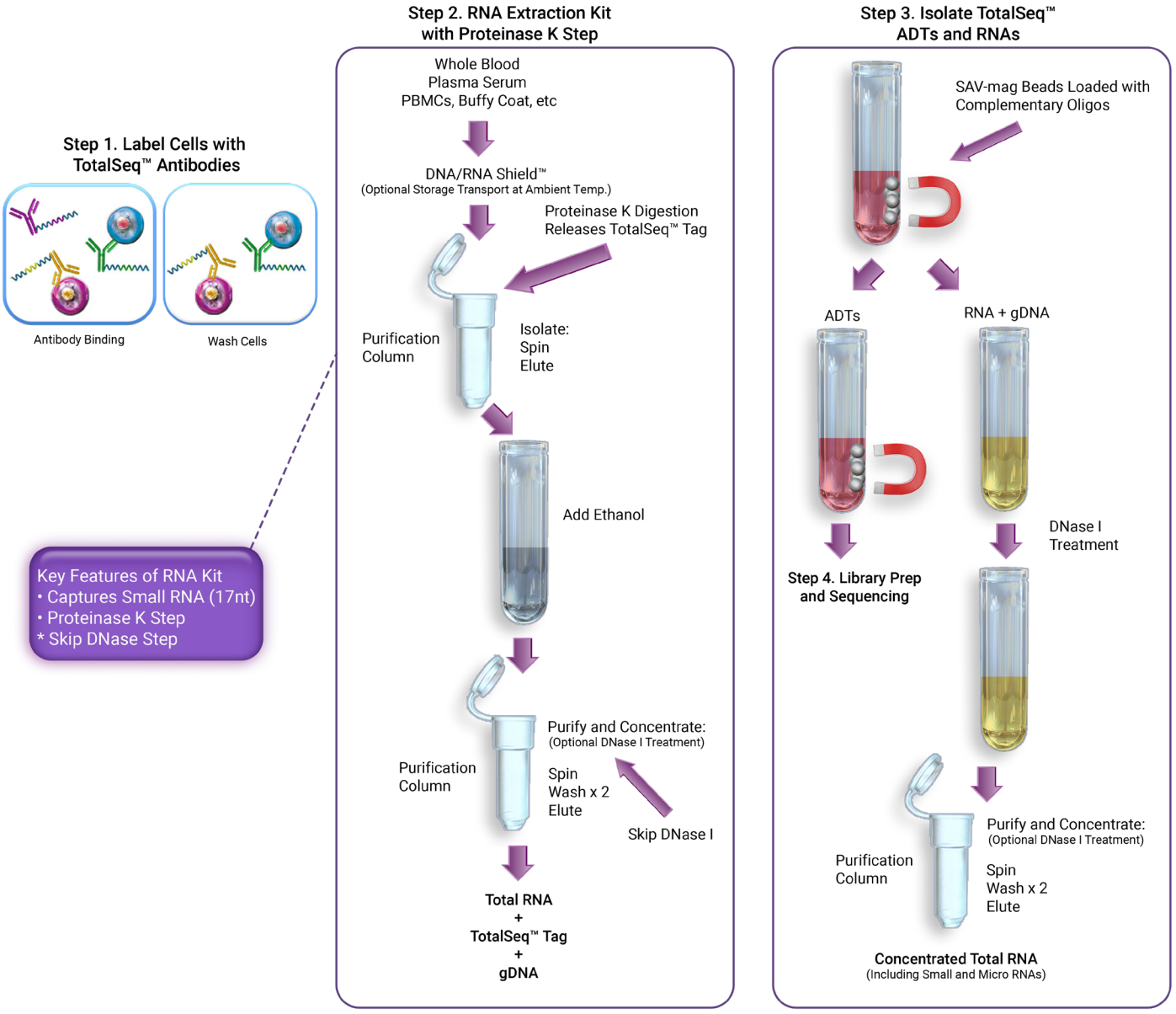


Learn more: biolegend.com/en-us/totalseq/mas

Bulk Epitope and Nucleic Acid Sequencing (BEN-seq)

Bulk RNA-sequencing is commonly used to analyze RNA expression from a heterogeneous cell population or tissue sample. Traditionally, this method has lacked the capacity to simultaneously measure proteins or the resolution to detect more than a few proteins in one experiment. While single-cell sequencing protocols have been adapted to include protein detection by sequencing, similar methods have not been developed for whole cell populations.

Our BEN-seq workflow, developed in collaboration with Illumina, stains cells in suspension using TotalSeq™-A antibodies prior to bulk RNA sequencing, enabling the simultaneous profiling of cell surface proteins and RNA.



Find protocols and download an application note: biolegend.com/en-us/totalseq/ben-seq

Single-Cell Protein and DNA Detection

Resolve complex genetic questions from genotype to phenotype by combining single-cell DNA analysis with protein detection using TotalSeq™-D oligo-conjugated reagents. Single-cell sequencing increases the sensitivity and enables the resolution of different genotypes within a heterogenous sample, which is critical for understanding complex diseases like cancer.

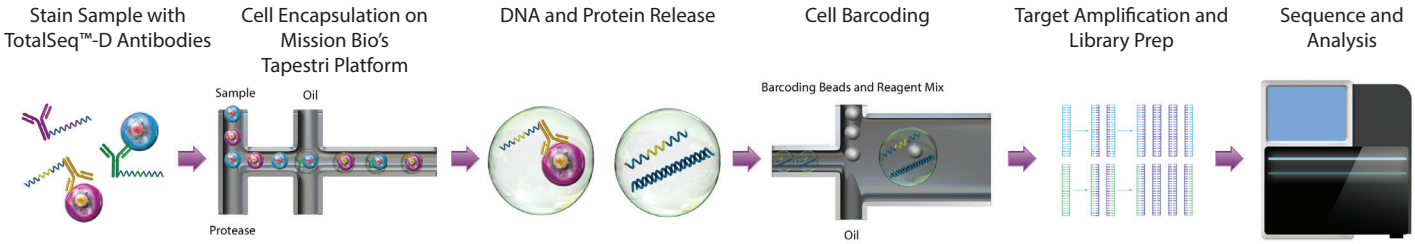
Resolution is increased by the addition of protein expression measurements and allows you to:

- Perform simultaneous immunophenotyping to determine whether mutations are associated with specific cell types or states.
- Discover new targets.
- Link genotype to phenotype by co-detecting SNVs/indels, CNVs, and proteins simultaneously.

Uncover genetic variations in heterogenous samples using Mission Bio's Tapestri platform and add BioLegend's TotalSeq™-D antibodies to correlate these mutations with protein expression.

Learn more: biolegend.com/en-us/totalseq/single-cell-dna

Single-Cell Protein and DNA Detection Workflow



Antibody Cocktails

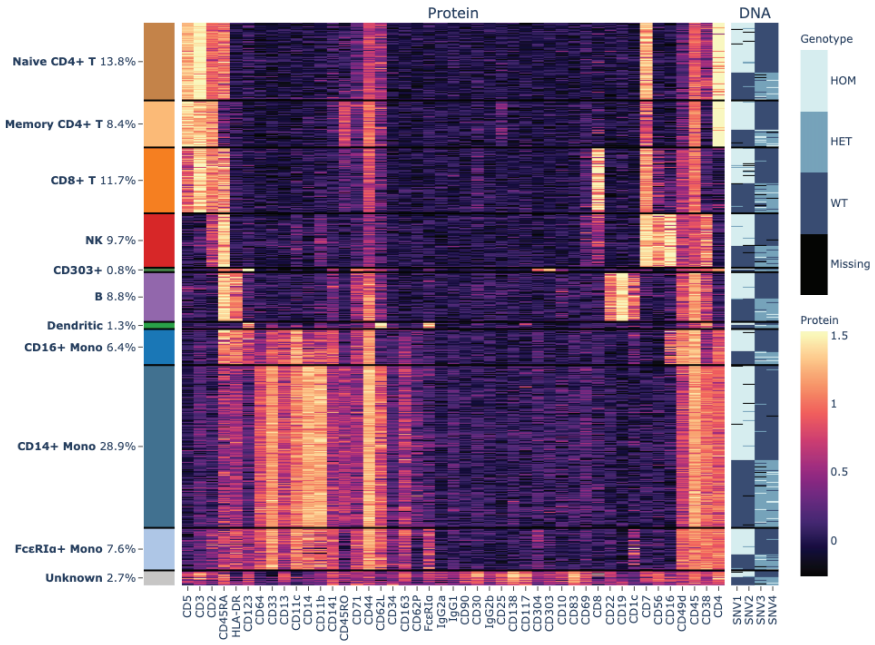
The TotalSeq™-D line of reagents is constructed for compatibility with the Mission Bio Tapestri platform. Each antibody is conjugated to an oligonucleotide that consists of a capture sequence, clone-specific barcode sequence, and a PCR handle compatible with Illumina® sequencing platforms.

Our TotalSeq™-D Heme Oncology Cocktail v1.0 contains 42 primary antibodies and 3 isotype control antibodies. Each antibody has been pre-titrated for optimal performance and the cocktail is provided in convenient, single-use tubes.

TotalSeq™-D Heme Oncology Cocktail v1.0 Targets:

CD1c	CD10	CD22	CD45	CD64	CD138
CD2	CD11b	CD25	CD45RA	CD69	CD141
CD3	CD11c	CD30	CD45RO	CD71	CD163
CD4	CD13	CD33	CD49d	CD83	CD303
CD5	CD14	CD34	CD56	CD90	CD304
CD7	CD16	CD38	CD62L	CD117	FcεRIα
CD8	CD19	CD44	CD62P	CD123	HLA-DR

Protein Cluster Signature vs Analyte and Barcode, Subsorted by DNA



PBMCs from two donor samples were mixed together, stained with TotalSeq™-D antibodies and run on Mission Bio's Tapestri platform. Heatmap visualization reveals the power to obtain genotype and phenotype from the same cells across thousands of cells.

Resources

Videos and Webinars

Watch introductory videos, protocol tutorials, and seminars to help you get started and learn more about how TotalSeq™ reagents can add value to your research.

Webinars Include:

- Simultaneous Proteomics and Transcriptomics Powered by TotalSeq™ Comprehensive Multiomics Solutions for T and B Cells
- Multimodal Techniques for High Content and High Throughput Cellular Phenotyping
- Getting a Grasp on Multimodal Single-Cell Omics Data
- Single-Cell Biology of Barrier Tissues and COVID-19

Watch now: biolegend.com/en-us/video-library

Blogs and Articles

Read our blog to learn more about single-cell multiomics, including tips and tricks for titrating antibodies, the basics of CITE-seq, and an overview of single-cell isolation methods.

Read now: biolegend.com/en-us/blog

eBook and Application Notes

Download our eBook and application notes to understand about the utility of TotalSeq™ reagents in multiomics applications.

- eBook: The Evolution and Future of Single-Cell Proteogenomics
- Correlated Expression of Protein and RNA Using Bulk and Single-Cell Proteogenomics
- Single-Cell Multiomics Reveals Novel Correlations Between Genomics Variants and Protein Expression in AML Patient Samples

Download today: biolegend.com/en-us/literature

Protocols

View our technical protocols for step-by-step instructions outlining how to use our TotalSeq™ reagents within sequencing workflows.

View now: biolegend.com/en-us/technical-protocols

Highlighted Publications

Multi-Omics Resolves a Sharp Disease-State Shift Between Mild and Moderate COVID-19

Su, Yapeng *et al. Cell,* 183:6, 1479 – 1495 (2020).

Using a panel of over 190 TotalSeq™ antibodies, this study used CITE-seq to identify novel immune cell populations that emerge in patients experiencing moderate COVID-19 that are expanded in severe cases.

A Conserved Dendritic Cell Regulatory Program Limits Antitumour Immunity

Maier, B. *et al. Nature,* 580, 257–262 (2020).

Immune checkpoint blockade is a recent cancer treatment method that induces a durable antitumor response. This study aims to understand the mechanism by which enhancement of systemic antitumor T cell immunity occurs after neoadjuvant PD-L1 blockade. Through the use of single-cell proteogenomics, the authors identify a dendritic cell regulatory program that limits antitumor activity and is induced by expression of IL-4.

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BioLegend products are manufactured in an ISO 13485:2016-certified facility to ensure the highest quality standards.

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