BioLegend
From Revvity

Multiomics with TotalSeq reagents

Antibodies, cocktails, and hashtags

revvity



Multiomics has revolutionized conventional sequencing experiments by enabling researchers to explore not just DNA or RNA expression in isolation, but also multiple modalities simultaneously, such as protein expression. TotalSeq™ oligo-conjugated antibodies have been instrumental in facilitating the analysis of cellular protein expression through sequencing, seamlessly integrating into both single-cell and bulk RNA and DNA sequencing workflows. Our extensive product portfolio includes antibodies, cocktails, cell hashtags for sample multiplexing, and an innovative single-cell protein-only assay designed to enhance protein detection via sequencing. Discover the capabilities of TotalSeq reagents and how they integrate effortlessly into established and cutting-edge workflows, revealing the distinctive characteristics of each individual cell.

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TotalSeq-A, -B, and -C: single-cell protein and RNA detection

TotalSeq oligo-conjugated antibodies enable detection of proteins at a single-cell level in applications that incorporate simultaneous measurement of both gene and protein expression, such as CITE-Seq. They integrate 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.

Benefits of using TotalSeq antibodies

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 as variants that 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 hundreds of proteins in a single cell. A lower dropout rate contributes to enhanced sample clustering and the ability to better identify specific cell types.

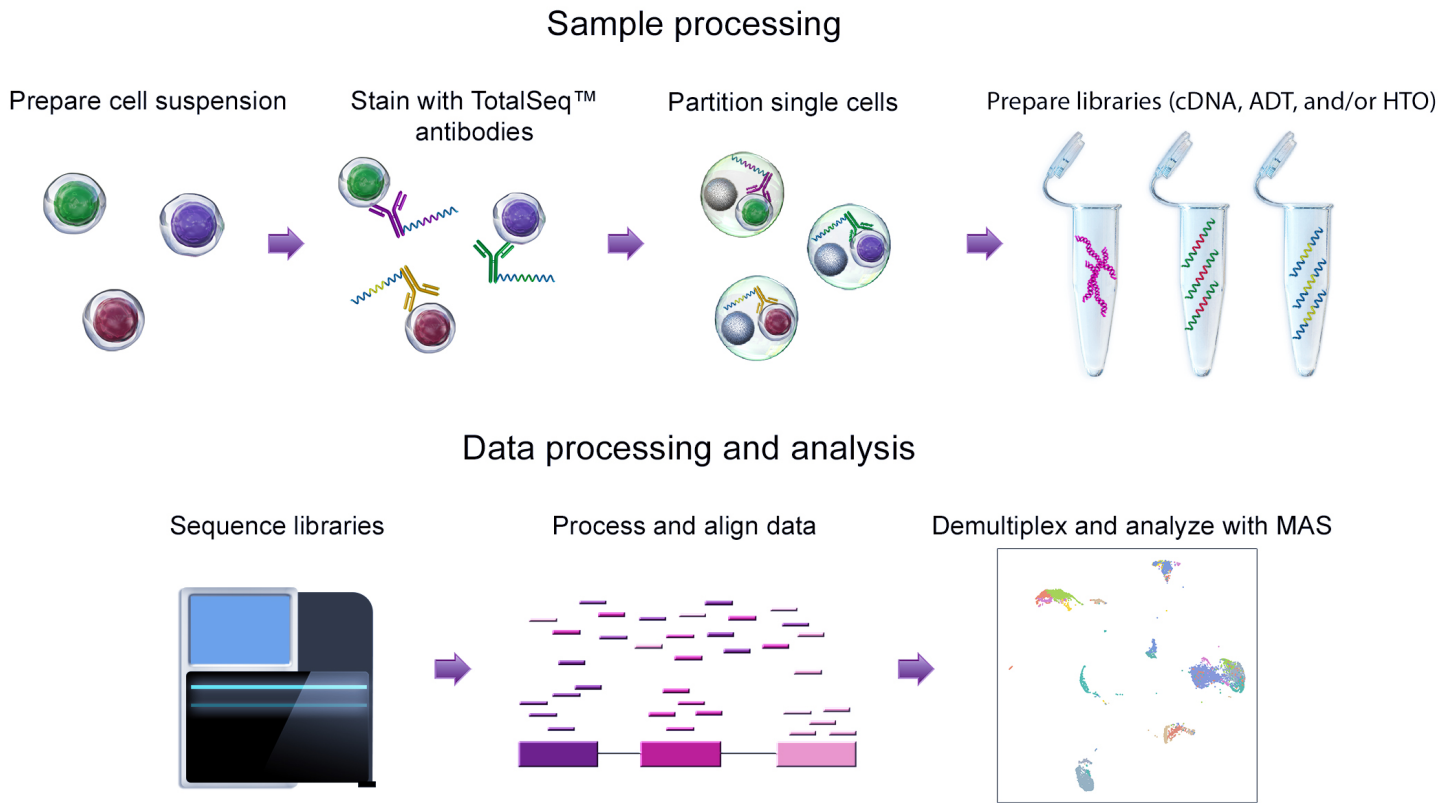
Increased efficiency: Easily combine multiple samples with our cell hashing antibodies to improve workflows and reduce experimental cost.

Simultaneous multiomic data generation: Increase the power of single-cell experiments by combining proteomic and transcriptomic data.

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

Simultaneous single-cell protein and RNA detection workflow



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

Antibody formats for single-cell protein and RNA analysis

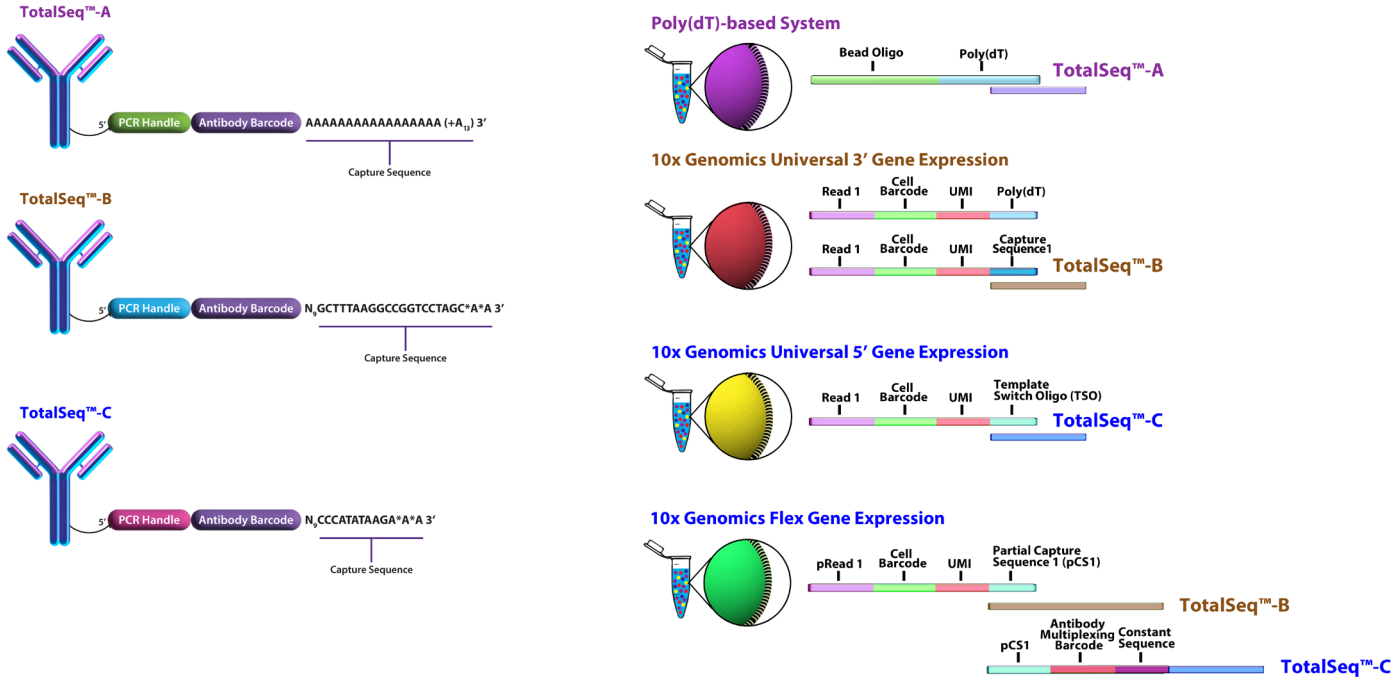
Leveraging our portfolio of trusted antibody clones, we offer the largest selection of oligo-barcoded antibodies. Antibodies are available in multiple formats so you can design customized experiments to answer your unique research questions. Each TotalSeq antibody is conjugated to a unique oligonucleotide containing a capture sequence, clone-specific barcode sequence, and PCR handle compatible with 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: This format contains a poly (A) capture sequence that mimics a natural mRNA. Designed to work with platforms that utilize poly (dT) oligonucleotides as the mRNA capture method. Compatible with 10x Genomics 3′ Universal Gene Expression assay, captured via the poly (dT) sequence of the Gel Bead primers. Use of TotalSeq-A reagents with this assay requires reagent and protocol modifications; 10x Genomics provides only limited support for TotalSeq-A. For additional information regarding the use of TotalSeq-A reagents, please refer to our protocols or contact BioLegend Technical Support.

TotalSeq-B: The capture sequence is compatible with the Capture Sequence 1 or Partial Capture Sequence 1 of the barcoded Gel Bead primers used with the 10x Genomics 3′ Universal Gene Expression or Flex Gene Expression assays, respectively. Use of TotalSeq-B antibodies with these assays is fully supported by 10x Genomics.

TotalSeq-C: The capture sequence is compatible with the Template Switch Oligo Capture Sequence or the Partial Capture Sequence 1 of the barcoded Gel Bead primers used with the 10x Genomics 5′ Universal Gene Expression or Flex Gene Expression assays, respectively. The use of TotalSeq-C antibodies with these assays is fully supported by 10x Genomics.



Compare and contrast antibody formats

	TotalSeq-A	TotalSeq-B	TotalSeq-C
10x Genomics Compatibility	Chromium Single Cell Universal 3′ Gene Expression assay and any system employing poly (A) tail	Chromium Single Cell Universal 3′ Gene Expression and Flex Gene Expression assays	Chromium Single Cell Universal 5′ Gene Expression and Flex Gene Expression assays
PCR handle	CCTTGGCACCCGAGAATTCCA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNN	CGGAGATGTGTATAAGAGACAGNNNNNNNNNN
Capture sequence	Poly-A [(A)30*A*A]	NNNNNNNNNGCTTTAAGGCCGGTCCTAGC*A*A	NNNNNNNNNCCCATATAAGA*A*A
Next-generation sequencing compatibility	Compatible with Illumina instruments	Compatible with Illumina instruments	Compatible with Illumina instruments

Notes:

N represents a randomly selected A, C, G, or T.

The symbol * indicates a phosphorothioated bond to prevent nuclease degradation.

TotalSeq-A,-B, and -C, antibodies are all compatible with 10x Genomics' Solutions, but 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.

TotalSeq-B and -C: intracellular protein staining

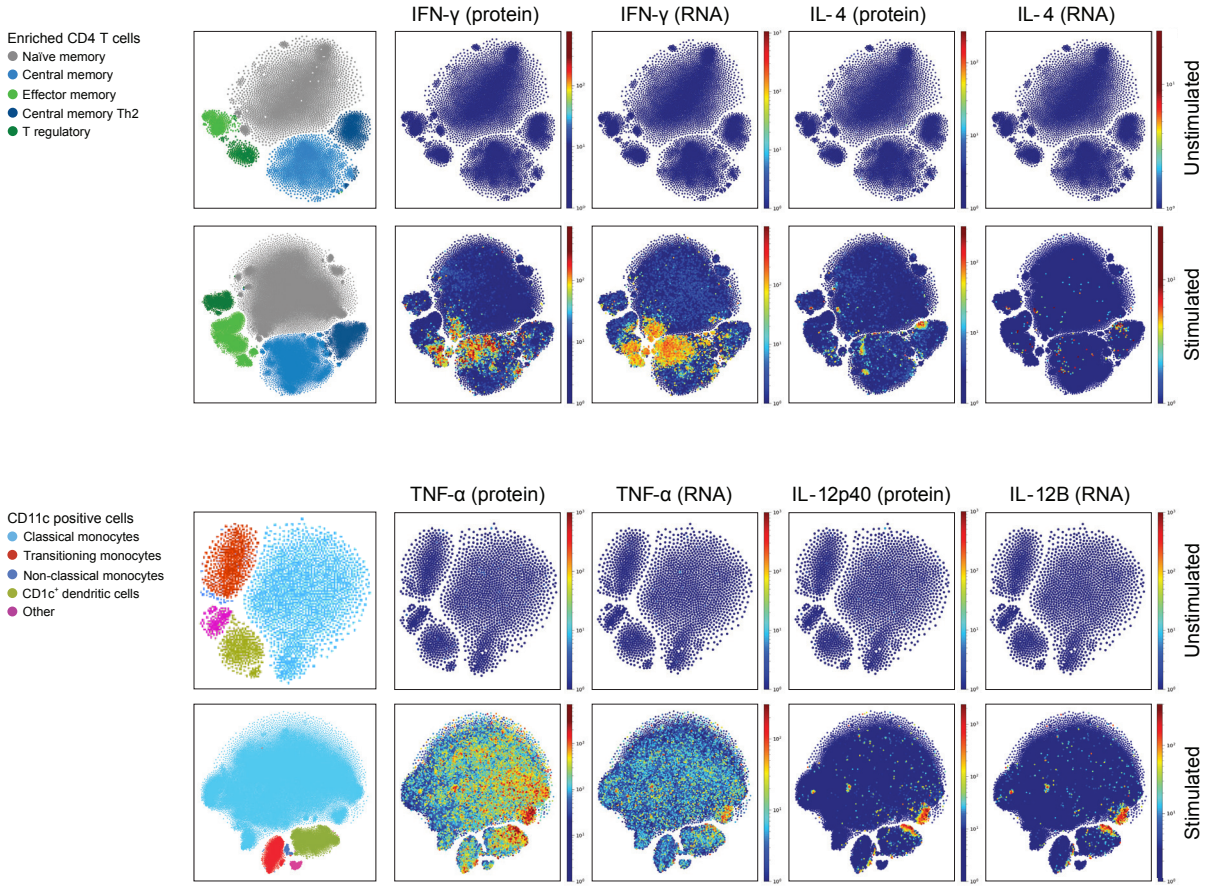
Intracellular proteins contribute to a host of cellular functions including cell growth, gene regulation, chemotaxis, and immunomodulation. Measuring both intracellular and surface proteins at the single-cell level can give researchers a comprehensive understanding of the cell and the molecular processes that underlie cellular function. The ability to detect cell surface proteins with gene expression in single-cell analysis has been well established using CITE-Seq. However, detecting intracellular proteins while preserving RNA quality for gene expression profiling has proven challenging, due largely to the conditions utilized to access the intracellular compartment of cells which can have deleterious effects on RNA quality.

With advancements in single-cell RNA technology and workflow optimization, it is now possible to observe all three modalities using TotalSeq antibodies and our TotalSeq-B or -C Cell Surface and Intracellular Protein Staining protocols that are compatible with 10x Genomics Chromium Single Cell Flex Gene Expression assays.

Why add intracellular targets to your single-cell experiment?

- Improve cell characterization by identifying functional molecules.
- Add depth to cell state identification by understanding signaling pathways.
- Discover unique and new phenotypes within seemingly homogeneous cell populations.

Intracellular and surface staining representative data



(Top) CD4+ T cells were magnetically enriched from PBMCs and stimulated with PMA and Ionomycin for six hours in the presence of BFA. (Bottom) CD14+ cells were magnetically enriched from PBMCs and stimulated with GM-CSF overnight in the presence of LPS and R848, and for the final six hours with Brefeldin A.

Sample multiplexing with hashtags

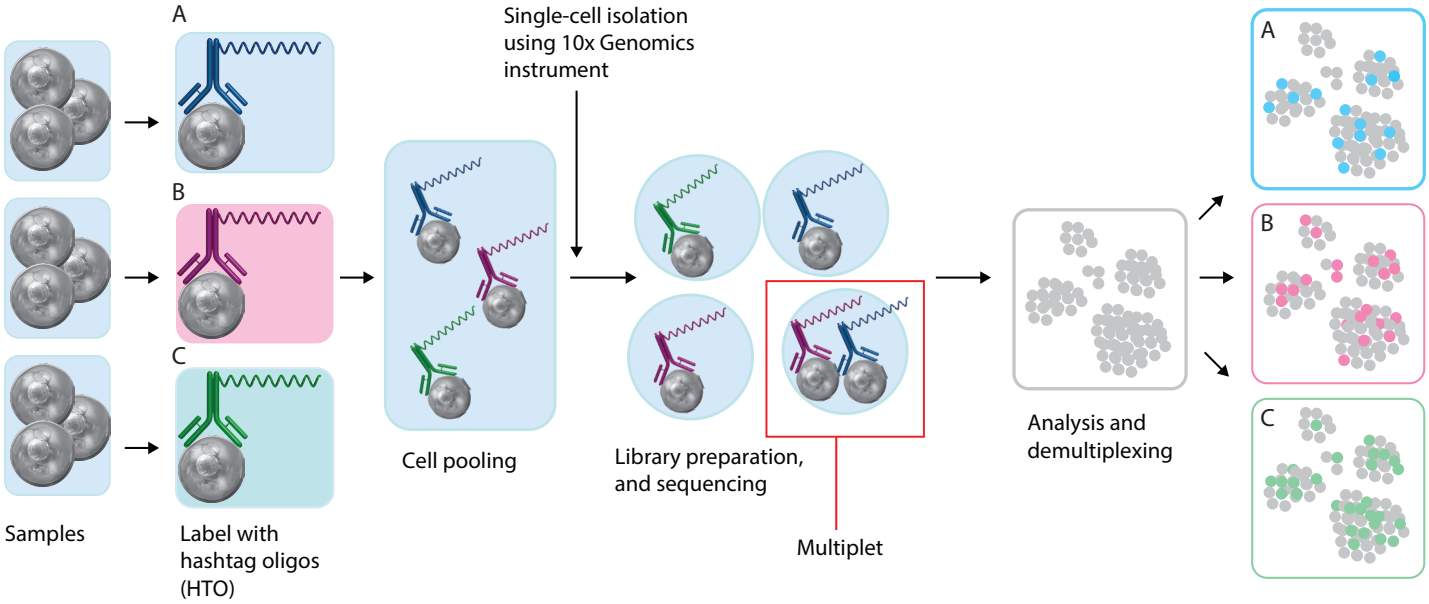
Cell hashing

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.

Hashtag workflow



Cell hashing workflow

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:

- 24 human and 24 mouse hashing antibodies available.
- ADT and HTO libraries are independently constructed, which can allow for further sequencing optimization.

TotalSeq-B and –C Hashtag reagents:

- 24 human and 24 mouse hashing antibodies available in each format.
- ADT and 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

Characterize immune cells to an unprecedented level

Gain flexible cellular phenotyping with our TotalSeq antibody cocktails. These pre-defined cocktails integrate with various single-cell RNA and DNA sequencing workflows, allowing you to analyze from nine to hundreds of proteins simultaneously, depending on your experimental goals.

Level of complexity and antibody multiplexing

Basic immunophenotyping	Targeted immunophenotyping	Deep and exploratory phenotyping
Human TBNK Cocktails	Human Essential Cocktails Mouse Myeloid Cocktail	Mouse and Human Universal Cocktails

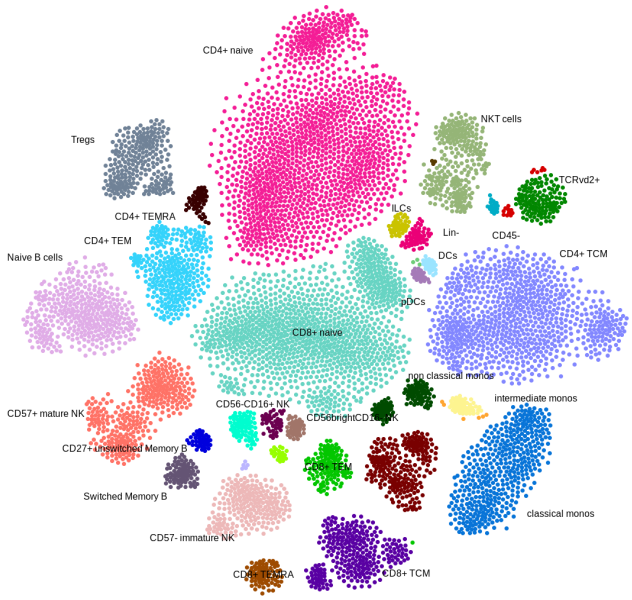
Deep phenotyping and exploratory cocktails

Universal Cocktails

TotalSeq Human and Mouse Universal Cocktails contain up to 175 primary antibodies for large-scale protein detection. These cocktails were designed to facilitate deep immunophenotyping, exploratory, or biomarker discovery applications. Our newest version 2.0 cocktails feature expanded content for greater dimensionality and include more antibodies pertinent to translational research and immuno-oncology applications.

Thirty-nine lymphoid lineage cell types were identified using the TotalSeq-C Human Universal Cocktail, V2.0. PBMCs were stained with the TotalSeq-C Human Universal Cocktail, V2.0 and processed using the 10x Genomics Single Cell 5′ v2 feature barcoding kit and Illumina sequencing. Sample file sets were imported into BioLegend MAS for final data analysis. Cell types were established using cell surface proteins for identification of various lymphoid lineage cell types. The expression of surface markers are shown on a novel cell type weighted t-distributed Stochastic Neighbor Embedding plots (weighted tSNE).

TotalSeq-C Human Universal Cocktail, V2.0



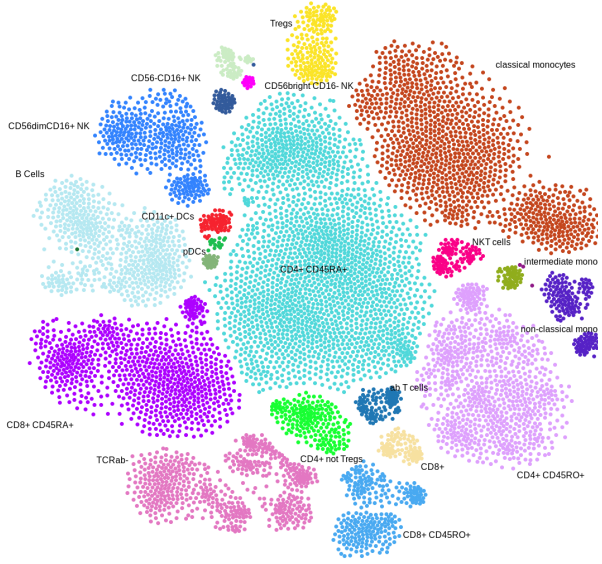
Targeted cocktails

Essential Cocktails

The TotalSeq Essential Cocktails contain 100 of the most frequently cited antibodies for characterizing principal and sub-lineage immune cell types. These cocktails offer a more focused phenotypic characterization while still providing a high multiplexing capacity, and they also include antibodies highly relevant to translational and immuno-oncology research.

Twenty-nine lymphoid lineage cell types were identified using the TotalSeq-C Human Essential Cocktail, V1.0. PBMCs were stained with the TotalSeq-C Human Essential Cocktail, V1.0 and processed using the 10x Genomics Single Cell 5′ v2 feature barcoding kit and Illumina sequencing. Sample file sets were imported into BioLegend MAS for final data analysis. Cell types were established using cell surface proteins for identification of various lymphoid lineage cell types. The expression of surface markers are shown on a novel cell type weighted t-distributed Stochastic Neighbor Embedding plots (weighted tSNE).

TotalSeq-C Human Essential Cocktail, V1.0



Mouse Myeloid Cocktail

The TotalSeq-B Mouse Myeloid Cocktail contains 76 primary antibodies to aid in the multiomic characterization of myeloid lineage cell types, and is ideal for samples depleted of lymphoid lineage cells. This cocktail has been optimized on enzymatically digested mouse splenocytes.

Human Heme Oncology Cocktail

The TotalSeq-D Human Heme Oncology Cocktail contains 42 primary antibodies. This cocktail was developed for compatibility with the Mission Bio Tapestri platform to simultaneously assess cell surface protein expression and unravel complex genotype-phenotype relationships in thousands of single cells in cancer research and related applications.

Basic immunophenotyping

TBNK Cocktails

Our TBNK Panels are designed to react with 9 primary antibodies for the identification of T, B, NK cells, and monocytes as defined by differential expression of CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD45, and CD56. These cocktails are ideal for a more accurate identification of principal lineage cell types, improved clustering, and initial CITE-Seq protocol standardization. They can serve as a base cocktail to spike-in additional content.

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.

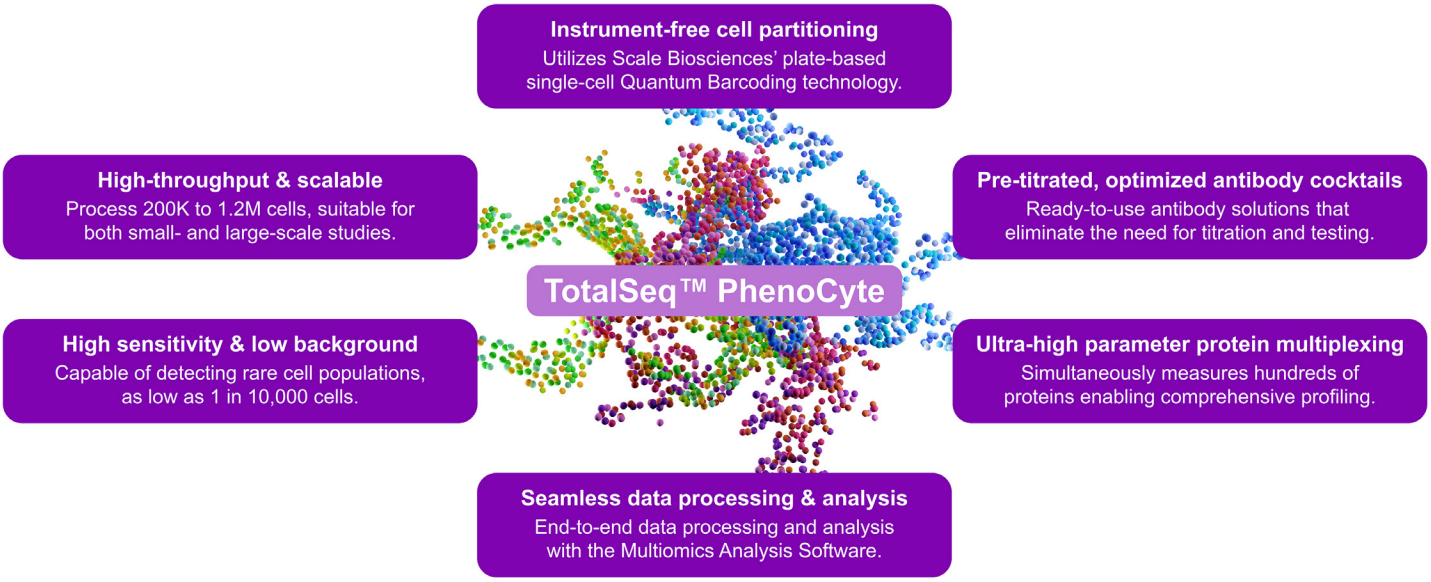
Our BEN-Seq workflow, developed in collaboration with Illumina, stains cells in suspension using TotalSeq-A antibodies prior to bulk RNA sequencing, enabling the simultaneous profiling of cell surface proteins and RNA.

Learn more about BEN-Seq at biolegend.com/en-us/totalseq/ben-seq

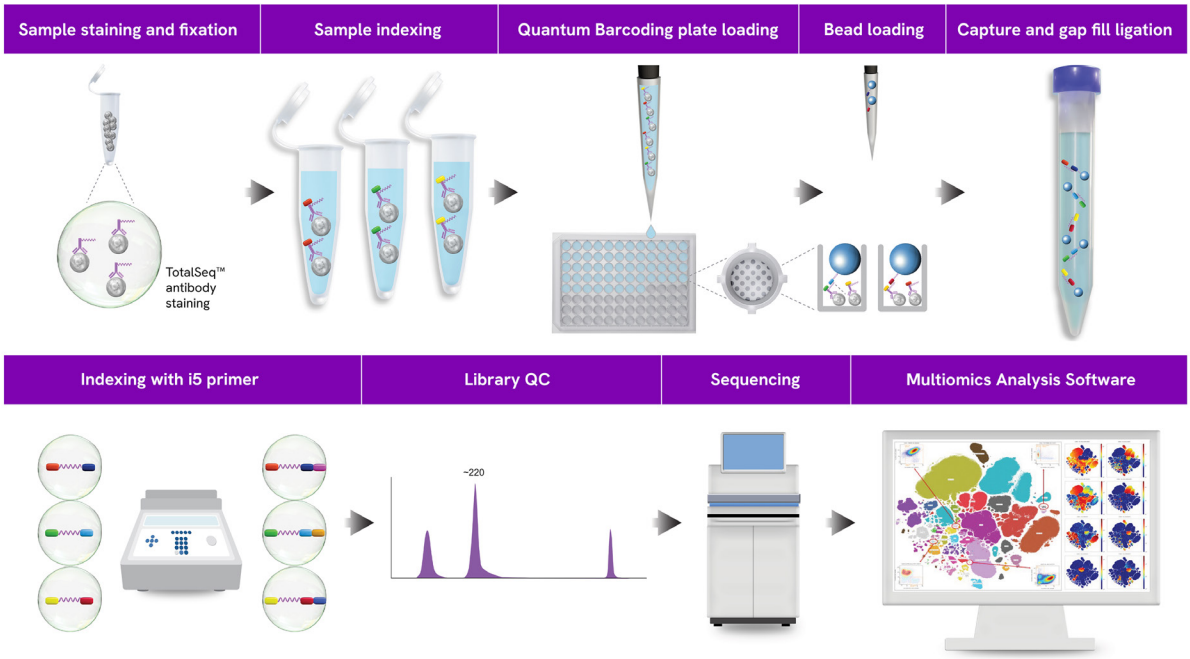
TotalSeq PhenoCyte: high-parameter single-cell protein analysis

Combining the power of our pre-titrated, optimized TotalSeq-A antibody panels with Scale Biosciences' Single-Cell Quantum Barcoding technology, TotalSeq PhenoCyte provides a simplified, instrument-free workflow for scalable single-cell immunoprofiling. Blending our expertise in flow cytometry and single-cell proteomics, this kit offers exceptional sensitivity, enabling high-throughput analysis with unparalleled sample multiplexing. TotalSeq PhenoCyte can help you streamline and amplify both the scale and scope of your single-cell research.

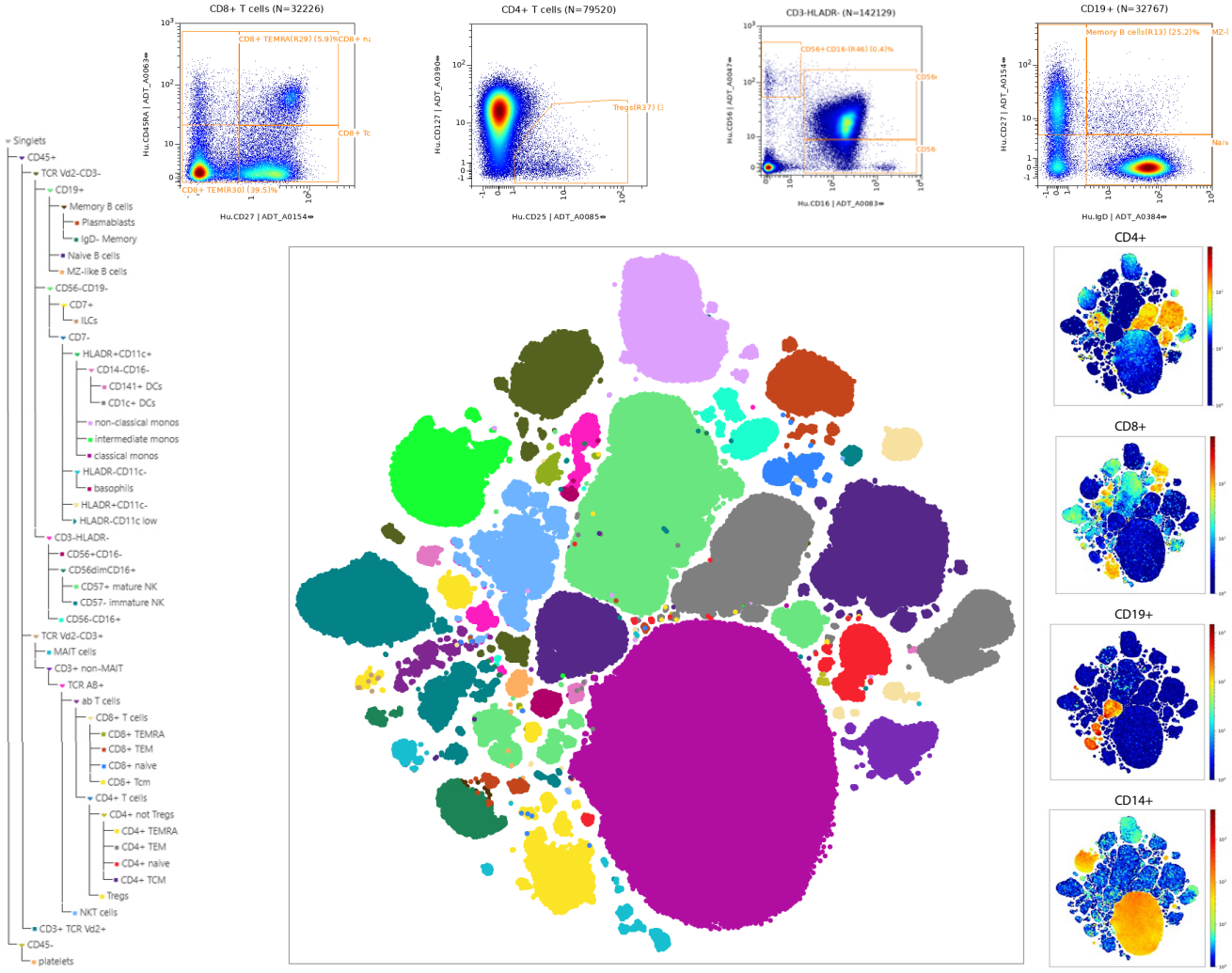
Learn more about TotalSeq PhenoCyte at biolegend.com/en-us/totalseq/phenocyte



TotalSeq PhenoCyte workflow overview



TotalSeq PhenoCyte data analysis



Resting PBMCs (approximately 1.2 million cells) were profiled using the TotalSeq-A PhenoCyte Human Universal V2.0 kit, enabling detection of 175 protein markers. Data analysis followed the TotalSeq PhenoCyte workflow and was performed using BioLegend's Multiomic Analysis Software. Displayed bivariate plots represent a select subset of the 51 total populations that were gated, with minimal background noise. A wt-SNE projection of all gated subpopulations is shown, alongside representative plots highlighting the expression patterns of select protein markers.

TotalSeq-D: single-cell protein and DNA detection

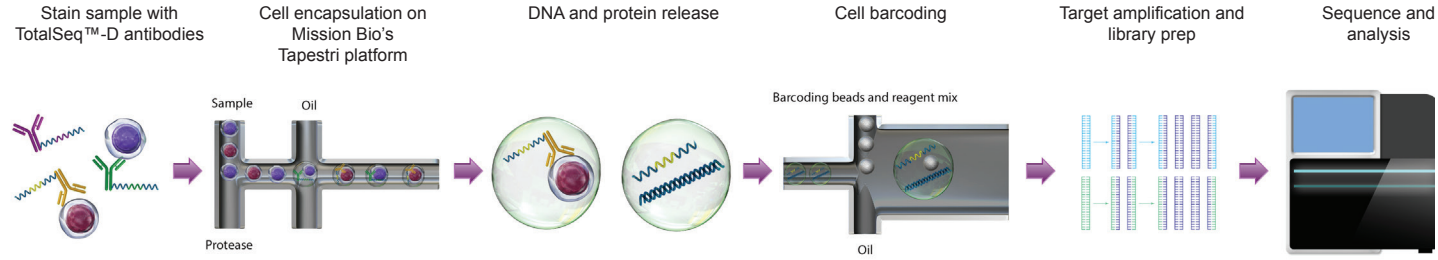
Resolve complex genetic questions from genotype to phenotype by combining single-cell DNA analysis with protein detection using TotalSeq-D oligo-conjugated reagents. Single-cell sequencing increases the sensitivity and enables the resolution of different genotypes within a heterogeneous sample, which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

Single-cell protein and DNA detection workflow



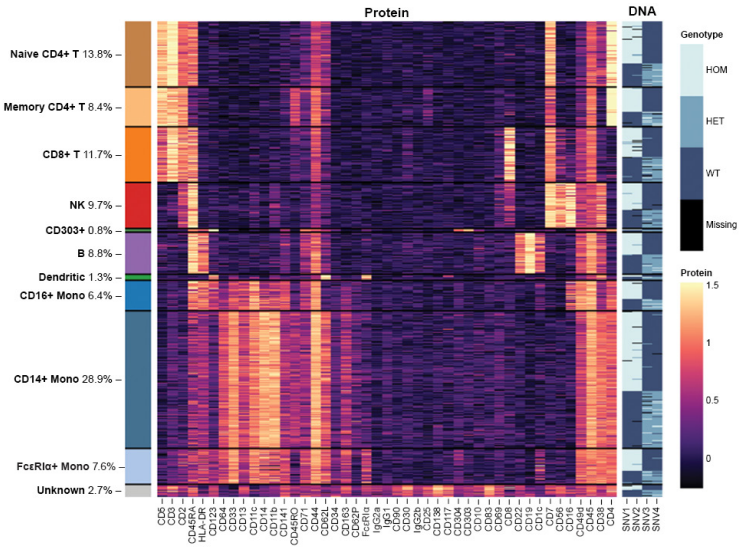
Antibody cocktails

Our TotalSeq-D Heme Oncology Cocktail v1.0 contains 42 primary antibodies and 3 isotype control antibodies. Each antibody is pre-titrated, provided in a single-use tube, and conjugated to an oligonucleotide consisting of a capture sequence, clone-specific barcode sequence, and a PCR handle compatible with Illumina® sequencing platforms.

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.

TotalSeq-D hashtag reagents

Multiplex up to three samples per Tapestri run using our TotalSeq-D Hashtag reagents. We have 3 human and 3 mouse hashtag antibodies available.

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

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.

Immunophenotype cells using flow-like gating

MAS's interactive interface allows you to immunophenotype cells with flow-like gating, offering a precise, intuitive approach to analyzing complex cellular populations.

Reveal hidden cell populations

MAS's clustering algorithms allow you to identify and distinguish unique cell subsets, even in complex and heterogeneous sampling, helping you uncover novel insights that could otherwise be missed with traditional methods.

Streamline data interpretation

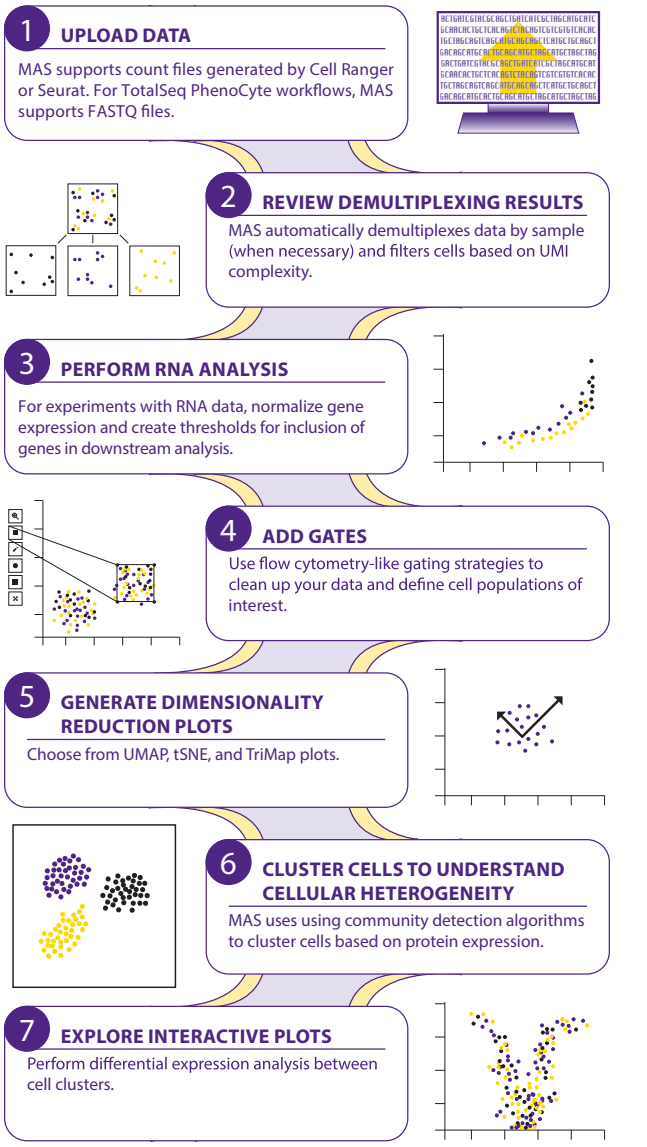
These visualization tools enable intuitive exploration of large, complex data sets, turning millions of data points into actionable insights.

Simplify complex data

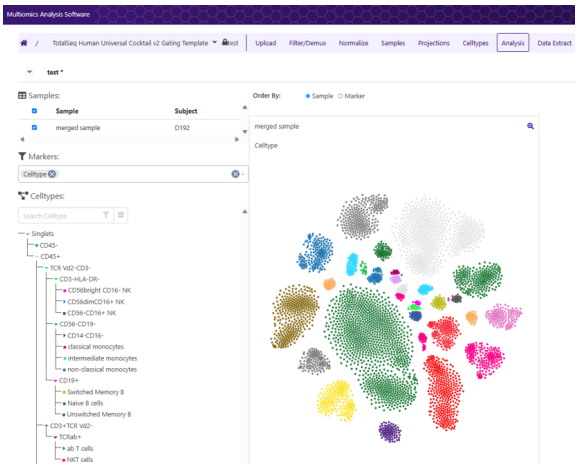
MAS can perform dimensionality reduction techniques such as weighted t-SNE and UMAP, transforming your data into intuitive, easy-to-read visualizations, making it straightforward to spot relationships, patterns, or anomalies between cells.

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

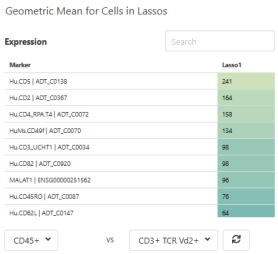
Multiomics Analysis Software Workflow



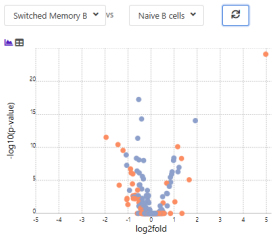
Create interactive plots for easy exploration of single-cell data



View protein and RNA expression data by cluster



Compare clusters to identify markers of interest



Visualize protein and RNA expression



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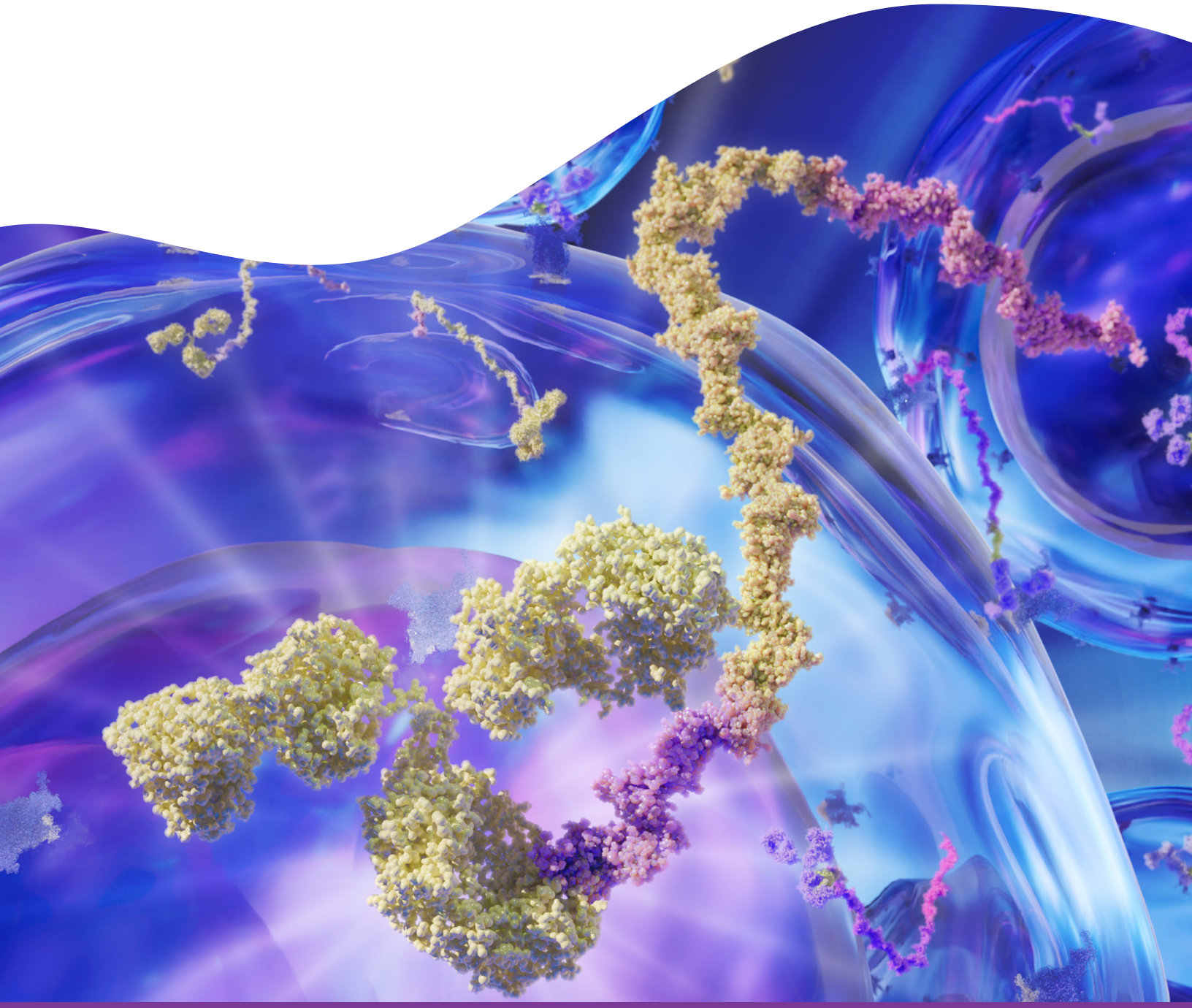

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