Store at -20°C

# InTraSeq™ 3' Conjugate Antibody Cocktail 1

#48167

22 µL
(4 assays)

**Support:** +1-978-867-2388 (U.S.)
cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)
orders@cellsignal.com

**For Research Use Only. Not for Use in Diagnostic Procedures.**

| Antibody Cocktail Includes | Product # | Reactivity | Isotype |
|---|---|---|---|
| Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3003) | 82746 | H, M | Rabbit IgG |
| S6 Ribosomal Protein (54D2) Mouse mAb (InTraSeq™ 3' Conjugate 3005) | 38889 | H, M | Mouse IgG1 |
| Phospho-CREB (Ser133) (87G3) Rabbit mAb (InTraSeq™ 3' Conjugate 3006) | 95580 | H, M | Rabbit IgG |
| GAPDH (14C10) Rabbit mAb (InTraSeq™ 3' Conjugate 3007) | 27907 | H, M | Rabbit IgG |
| CREB (48H2) Rabbit mAb (InTraSeq™ 3' Conjugate 3008) | 41320 | H, M | Rabbit IgG |
| CD19 (Intracellular Domain) (D4V4B) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3010) | 76014 | H, M | Rabbit IgG |
| Vimentin (D21H3) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3011) | 96524 | H, M | Rabbit IgG |
| TCF1/TCF7 (C63D9) Rabbit mAb (InTraSeq™ 3' Conjugate 3013) | 44070 | H, M | Rabbit IgG |
| Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3014) | 53639 | H, M | Rabbit IgG |
| Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3015) | 69098 | H, M | Rabbit IgG |
| p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (InTraSeq™ 3' Conjugate 3016) | 79941 | H, M | Rabbit IgG |
| Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb (InTraSeq™ 3' Conjugate 3017) | 53207 | H, M | Rabbit IgG |
| Akt (pan) (C67E7) Rabbit mAb (InTraSeq™ 3' Conjugate 3018) | 35123 | H, M | Rabbit IgG |
| Phospho-S6 Ribomosal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3019) | 92243 | H, M | Rabbit IgG |
| Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3020) | 27234 | H, M | Rabbit IgG |
| Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (InTraSeq™ 3' Conjugate 3021) | 45003 | H, M | Rabbit IgG |
| Stat3 (124H6) Mouse mAb (InTraSeq™ 3' Conjugate 3022) | 68474 | H, M | Mouse IgG2a κ |
| Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (InTraSeq™ 3' Conjugate 3023) | 76939 | H, M | Rabbit IgG |
| Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3024) | 92612 | H, M | Rabbit IgG |
| NCAM1 (CD56) (E7X9M) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3025) | 17655 | H, M | Rabbit IgG |
| Ikaros (D6N9Y) Rabbit mAb (InTraSeq™ 3' Conjugate 3026) | 42531 | H, M | Rabbit IgG |
| Aiolos (D1C1E) Rabbit mAb (InTraSeq™ 3' Conjugate 3027) | 61429 | H, M | Rabbit IgG |
| Phospho-Stat3 (Ser727) (D4X3C) Rabbit mAb (InTraSeq™ 3' Conjugate 3031) | 34774 | H, M | Rabbit IgG |

**Storage:** This product is stable for at least 12 months when stored at -20ºC.

**Please visit cellsignal.com for validation data and a complete listing of recommended companion products.**

**Description:** The InTraSeq™ methodology enables researchers to measure proteins (cytoplasmic, surface, and nuclear), including post-translational modifications (PTMs), along with RNA in their single-cell assays. While single-cell RNA sequencing (scRNAseq) has transformed the field of cellular and molecular biology, it only measures RNA transcripts within single cells. This presents a significant challenge, as RNA levels don't necessarily translate to protein abundance or activity. The InTraSeq technology addresses this limitation and can unravel missing information in scRNAseq experiments, uncovering new cell states and crucial biological data in single-cell analyses, especially since RNA does not reflect PTMs.

InTraSeq™ 3' Conjugate Antibody Cocktail 1 is a mixture of 31 titrated InTraSeq™ 3' Conjugates that are conjugated to 10x Genomics Feature Barcodes. This cocktail is only compatible with the InTraSeq™ 3' Assay Kit #82906 and is validated by CST using the 10x Genomics Chromium Single Cell 3' Kits with Feature Barcoding technology.

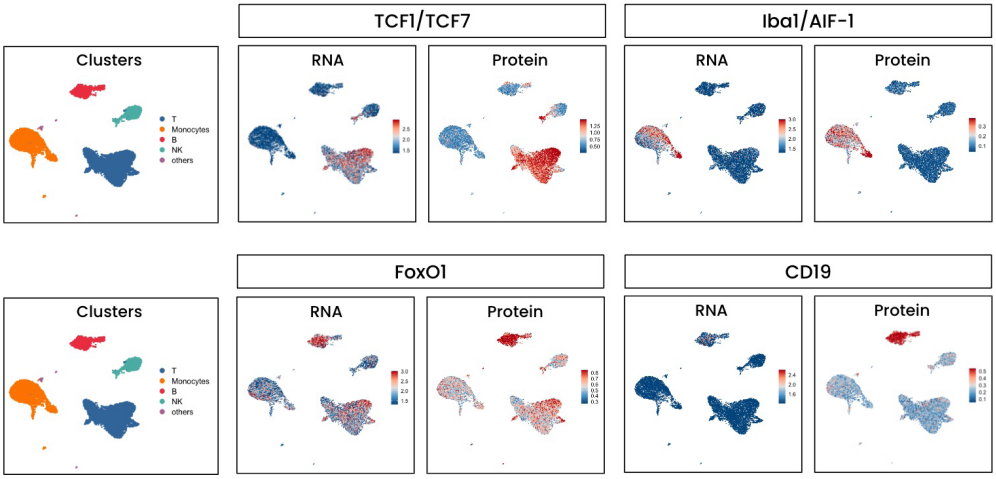




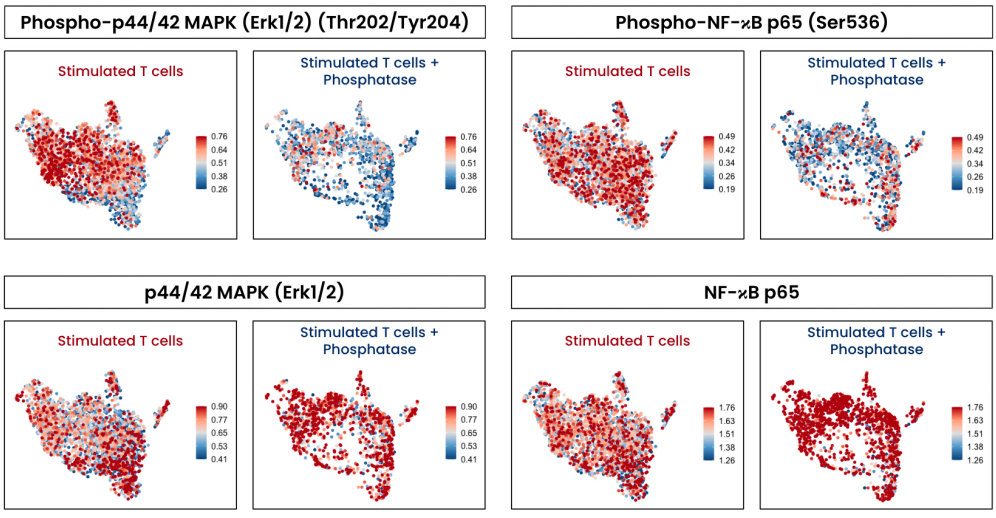


**Applications:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
**Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
**All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

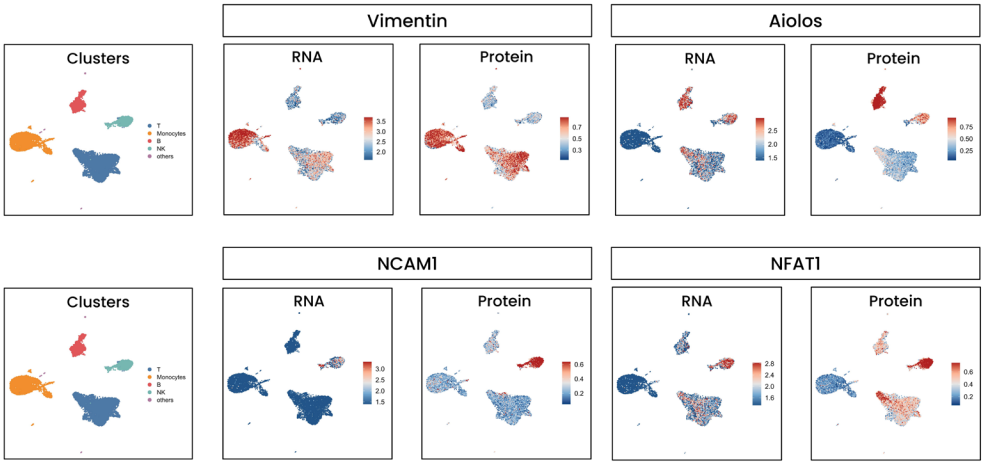
| Antibody Cocktail Includes (cont.) | Product # (cont.) | Reactivity (cont.) | Isotype (cont.) |
|---|---|---|---|
| NFAT1 (D43B1) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3032) | 53722 | H, M | Rabbit IgG |
| NF-κB p65 (D14E12) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3033) | 76695 | H, M | Rabbit IgG |
| Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (InTraSeq™ 3' Conjugate 3034) | 97410 | H, M | Rabbit IgG |
| Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3035) | 25504 | H, M | Rabbit IgG |
| Phospho-Glucocorticoid Receptor (Ser226) (D9D3V) Rabbit mAb (InTraSeq™ 3' Conjugate 3036) | 25665 | H, M | Rabbit IgG |
| FoxO1 (C29H4) Rabbit mAb (InTraSeq™ 3' Conjugate 3037) | 45300 | H, M | Rabbit IgG |
| Stat1 (D1K9Y) Rabbit mAb (InTraSeq™ 3' Conjugate 3038) | 41599 | H, M | Rabbit IgG |
| Phospho-Stat1 (Ser727) (D3B7) Rabbit mAb (InTraSeq™ 3' Conjugate 3039) | 82887 | H, M | Rabbit IgG |



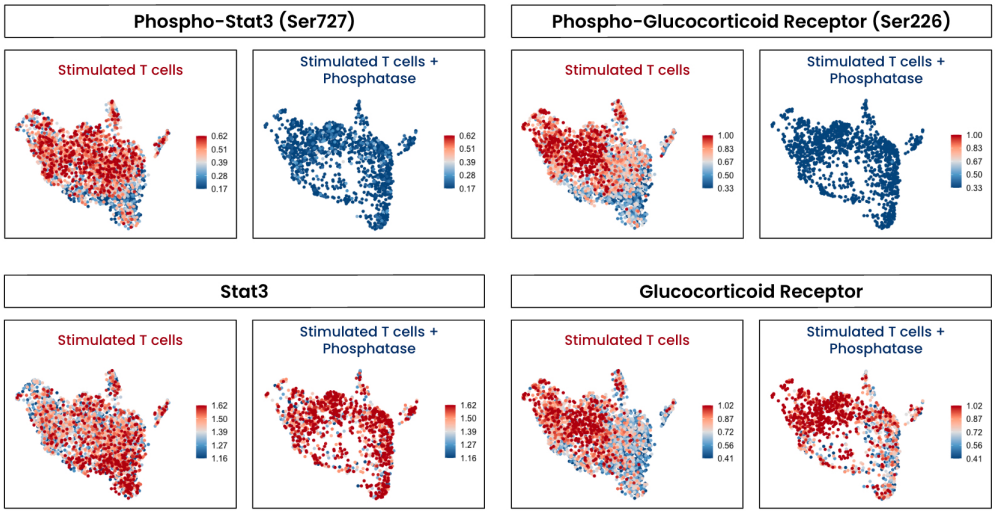

*Human peripheral blood mononuclear cells (PBMCs) were processed in accordance with the InTraSeq™ 3' protocol. The left-most figures show the Uniform Manifold Approximation and Projection (UMAP) of the annotated PBMC clusters in each row. The top left figures display FeaturePlots of the TCF7 RNA and TCF1/TCF7 (C63D9) Rabbit mAb (InTraSeq™ 3' Conjugate 3013) #44070 protein expression levels. The top right figures display FeaturePlots of the AIF1 RNA and Iba1/AIF-1 (E4O4W) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3003) #82746 protein expression levels. The bottom left figures display FeaturePlots of the FOXO1 RNA and FoxO1 (C29H4) Rabbit mAb (InTraSeq™ 3' Conjugate 3037) #45300 protein expression levels. The bottom right figures display FeaturePlots of the CD19 RNA and CD19 (Intracellular Domain) (D4V4B) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3010) #76014 protein expression levels. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q95", min.cutoff = "q5", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*



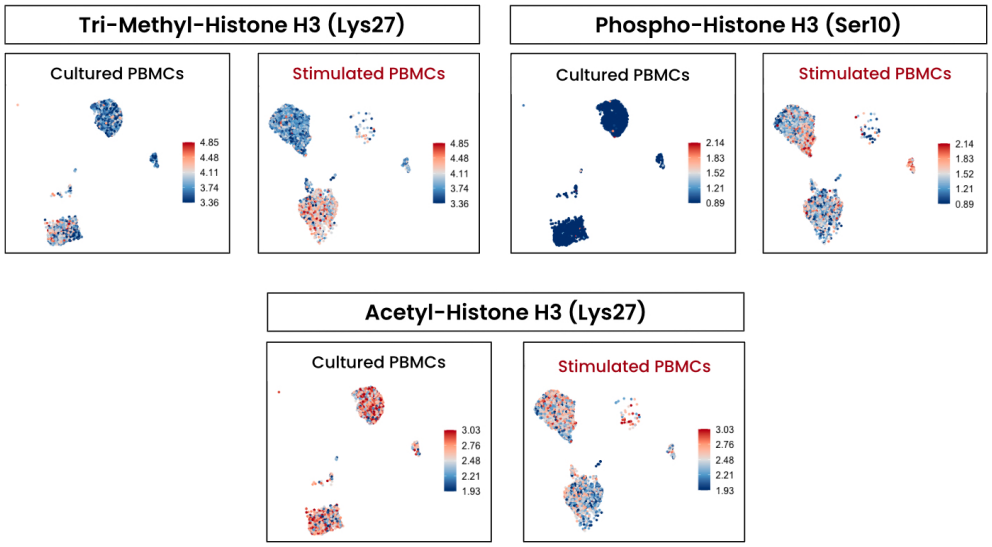

*Cultured human peripheral blood mononuclear cells (PBMCs) treated with Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min, Stimulated; left panels) or treated with Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min) then subsequently treated with Lambda Protein Phosphatase (40 min, 30°C, Stimulated + Phosphatase; right panels) were processed in accordance with the InTraSeq™ 3' protocol. The T cell population was then selected and analyzed. The FeaturePlots in the top left figures display the Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (197G2) Rabbit mAb (InTraSeq™ 3' Conjugate 3023) #76939 expression on the Uniform Manifold Approximation and Projection (UMAP). The FeaturePlots in the bottom left figures display the p44/42 MAPK (Erk1/2) (137F5) Rabbit mAb (InTraSeq™ 3' Conjugate 3016) #79941 expression on the UMAP. The FeaturePlots in the top right figures display the Phospho-NF-κB p65 (Ser536) (93H1) Rabbit mAb (InTraSeq™ 3' Conjugate 3034) #97410 expression on the UMAP. The FeaturePlots in the bottom right figures display the NF-κB p65 (D14E12) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3033) #76695 expression on the UMAP. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q95", min.cutoff = "q5", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*



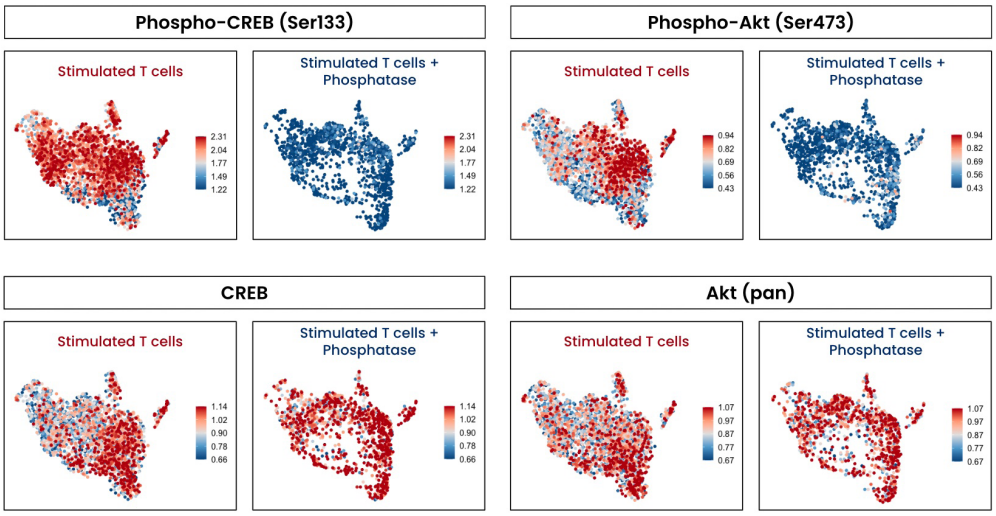

*Human peripheral blood mononuclear cells (PBMCs) were processed in accordance with the InTraSeq™ 3' protocol. The leftmost figures show the Uniform Manifold Approximation and Projection (UMAP) of the annotated PBMC clusters in each row. The top left figures display FeaturePlots of the VIM RNA and Vimentin (D21H3) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3011) #96524 protein expression levels. The top right figures display FeaturePlots of the IKZF3 RNA and Aiolos (D1C1E) Rabbit mAb (InTraSeq™ 3' Conjugate 3027) #61429 protein expression levels. The bottom left figures display FeaturePlots of the NCAM1 RNA and NCAM1 (CD56) (E7X9M) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3025) #17655 protein expression levels. The bottom right figures display FeaturePlots of the NFATC2 RNA and NFAT1 (D43B1) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3032) #53722 protein expression levels. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q95", min.cutoff = "q5", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*




*Cultured human peripheral blood mononuclear cells (PBMCs) treated with Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min, Stimulated; left panels) or treated with Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min) then subsequently treated with Lambda Protein Phosphatase (40 min, 30°C, Stimulated + Phosphatase; right panels) were processed in accordance with the InTraSeq™ 3' protocol. The T cell population was then selected and analyzed. The FeaturePlots in the top left figures display the Phospho-Stat3 (Ser727) (D4X3C) Rabbit mAb (InTraSeq™ 3' Conjugate 3031) #34774 expression on the Uniform Manifold Approximation and Projection (UMAP). The FeaturePlots in the bottom left figures display the Stat3 (124H6) Mouse mAb (InTraSeq™ 3' Conjugate 3022) #68474 expression on the UMAP. The FeaturePlots in the top right figures display the Phospho-Glucocorticoid Receptor (Ser226) (D9D3V) Rabbit mAb (InTraSeq™ 3' Conjugate 3036) #25665 expression on the UMAP. The FeaturePlots in the bottom right figures display the Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3035) #25504 expression on the UMAP. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q95", min.cutoff = "q5", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*




*Cultured human peripheral blood mononuclear cells (PBMCs) untreated (Cultured; left panels) or treated with Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min, Stimulated; right panels) were processed in accordance with the InTraSeq™ 3' protocol. The FeaturePlots in the top left figures display the Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb (InTraSeq™ 3' Conjugate 3017) #53207 expression on the Uniform Manifold Approximation and Projection (UMAP). The FeaturePlots in the top right figures display the Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3024) #92612 expression on the UMAP. The FeaturePlots in the bottom middle figures display the Acetyl-Histone H3 (Lys27) (D5E4) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3020) #27234 expression on the UMAP. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q99", min.cutoff = "q5", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*

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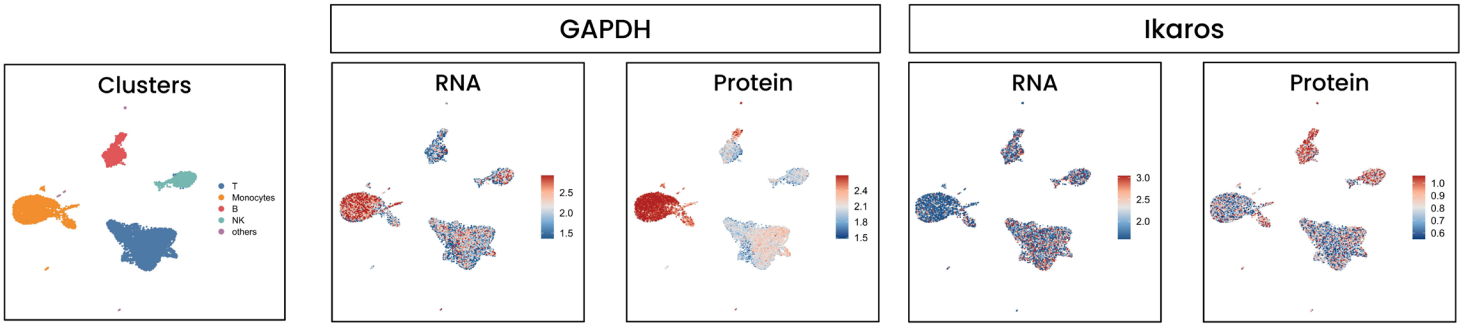
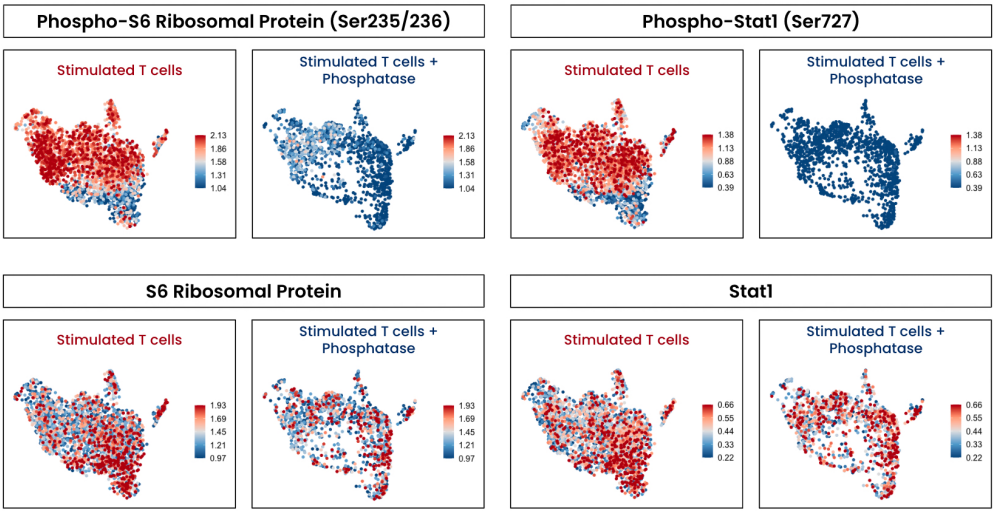



*Human peripheral blood mononuclear cells (PBMCs) were processed in accordance with the InTraSeq™ 3' protocol. The leftmost figure shows the Uniform Manifold Approximation and Projection (UMAP) of the annotated PBMC clusters. The middle figures display FeaturePlots of the GAPDH RNA and GAPDH (14C10) Rabbit mAb (InTraSeq™ 3' Conjugate 3007) #27907 protein expression levels. The right figures display FeaturePlots of the IKZF1 (Ikaros family zinc finger protein 1) RNA and Ikaros (D6N9Y) Rabbit mAb (InTraSeq™ 3' Conjugate 3026) #42531 protein expression levels. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q90", min.cutoff = "q10", order = FALSE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*

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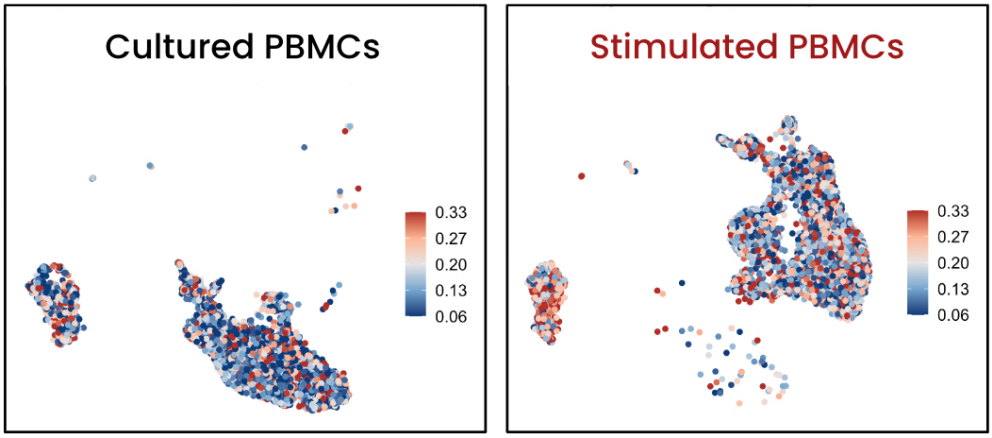
## Phospho-4E-BP1 (Thr37/46)




*Cultured human peripheral blood mononuclear cells (PBMCs) untreated (Cultured; left panel) or treated with Human IL-6 Recombinant Protein #48333, Human IL-2 Recombinant Protein #31058, Human IGF-I Recombinant Protein #29608, and Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min, Stimulated; right panel) were processed in accordance with the InTraSeq™ 3' protocol. The FeaturePlots in the figures display the Phospho-4E-BP1 (Thr37/46) (236B4) Rabbit mAb (InTraSeq™ 3' Conjugate 3021) #45003 expression on the Uniform Manifold Approximation and Projection (UMAP). To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q90", min.cutoff = "q10", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*

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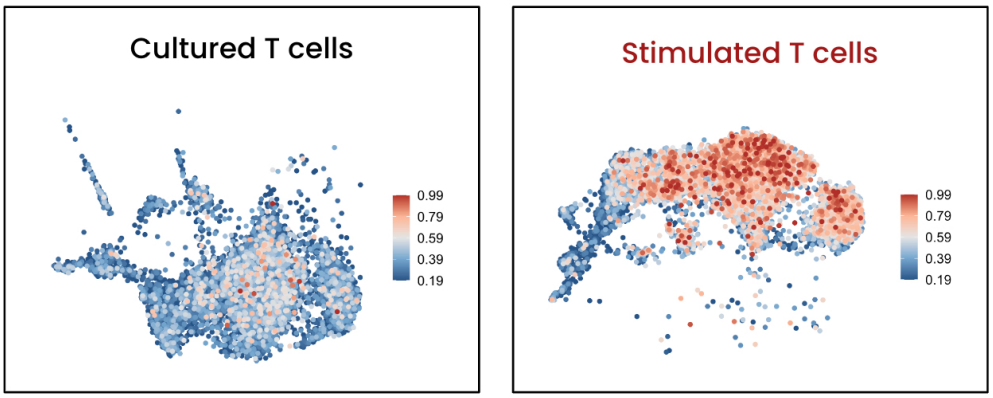
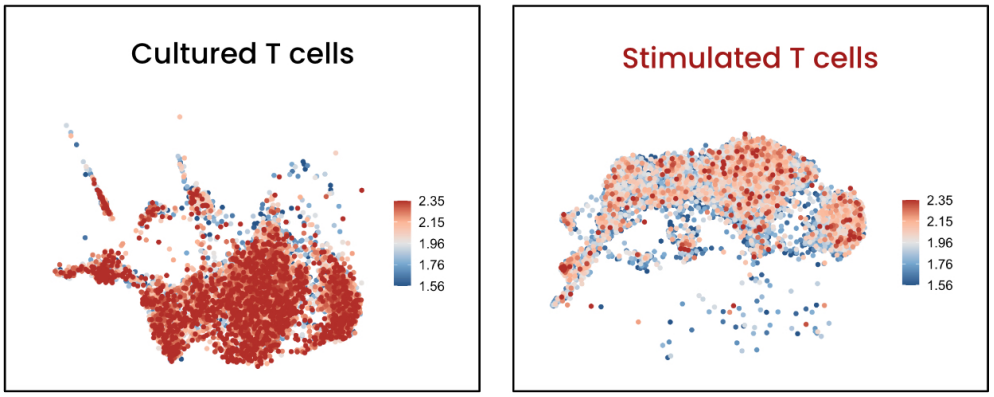



*Cultured human peripheral blood mononuclear cells (PBMCs) untreated (Cultured; left panels) or treated with Human IL-6 Recombinant Protein #48333, Human IL-2 Recombinant Protein #31058, Human IGF-I Recombinant Protein #29608, and Cell Stimulation Cocktail (without Protein Transport Inhibitors) (500X) #29255 (30 min, Stimulated; right panels) were processed in accordance with the InTraSeq™ 3' protocol. The T cell population was then selected and analyzed. The FeaturePlots in the top figures display the Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3015) #69098 expression on the Uniform Manifold Approximation and Projection (UMAP). The FeaturePlots in the bottom figures display the Stat3 (124H6) Mouse mAb (InTraSeq™ 3' Conjugate 3022) #68474 expression on the UMAP. To generate similar plots, use the FeaturePlot command template from the Seurat computational package: FeaturePlot(your_object, features = your_protein, max.cutoff = "q99", min.cutoff = "q1", order = TRUE) + scale_color_gradientn(colours = c("#22578B", "#73A9D2", "grey90", "#FDB393", "#b22222"))*

## Stat3



# InTraSeq™ 3' Protocol

## For best results:

- Maintain an RNase-free and sterile workspace.
- It is highly recommended to use filtered pipette tips.
- When starting section **B. Blocking (Day 2)** until the end of the protocol, it is crucial to perform the centrifugations at 850 x g (not RPM).
- When starting section **C. Immunostaining (Day 2)** in the protocol, it is crucial to maintain the cell pellet submerged (in ~40 µL) when removing and discarding the supernatant.
- Do not vacuum aspirate when discarding the supernatant in any step; always use a pipette.
- Make sure the reagents are completely thawed before use.

## NOTES:

- All InTraSeq™ 3' Assay Kit components are validated by CST in the 10x Genomics Chromium Single Cell 3' Kits with Feature Barcoding technology.
- **SAFE STOP –** This indicates a safe stopping point in the protocol if stopping is necessary.
- This protocol is designed for a single InTraSeq™ assay (one sample). If processing multiple samples, all quantities should be adjusted proportionally.

## Solutions and Reagents

Materials Included in Kit (Store at -20°C)

- InTraSeq™ Blocking Buffer A #10471
- InTraSeq™ Blocking Buffer B #31701
- InTraSeq™ Staining Buffer #46005
- InTraSeq™ Wash Buffer #59335
- InTraSeq™ Reducing Agent #63760
- InTraSeq™ RNase Inhibitor #79298
- Rabbit (DA1E) mAb IgG XP® Isotype Control (InTraSeq™ 3' Conjugate 3000) #81472
- Histone H3 (D1H2) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3002) #68984

## Required Reagents Not Included

- InTraSeq™ 3' Conjugates/Antibody Cocktails
- Two sterile 40-micron Falcon cell strainers
- Vortex mixer
- 1.5 mL Sterile Polypropylene Microcentrifuge Tubes - LoBind
- 15 mL Conical Sterile Polypropylene Centrifuge Tubes (preferably LoBind)
- 50 mL Conical Sterile Polypropylene Centrifuge Tubes (preferably LoBind)
- Phosphate Buffered Saline (PBS-1X) pH7.2 (Sterile) #9872
- Methanol #13604

## Before starting:

(Optional) When you receive the kit, thaw and pipette mix the InTraSeq™ Blocking Buffer A, then aliquot 870 µL into 8 separate 1.5 mL sterile microcentrifuge tubes.

## A. Cell Fixation (Day 1)

**NOTE:** If performing an immunostaining step prior to this protocol, we highly recommend beginning immunostaining with at least 2 million cells to guarantee 1-5 million cells in step 1 below.

1. Count and transfer 1-5 million cells to a 15 mL tube.

   **NOTE:** Do not proceed with the protocol with less than 1 million cells.
2. Centrifuge the cells at 300 x g for 5 min at 4°C.
3. Remove and discard the supernatant, then wash the cells with 10 mL of ice-cold 1X PBS.
4. Centrifuge the cells at 300 x g for 5 min at 4°C.
5. Remove and discard supernatant, without disturbing the cell pellet, then resuspend the cells in 0.5 mL of ice-cold 1X PBS.
6. Turn on the vortex mixer and keep it on the "**On**" switch (**not the "Auto/Touch" mode**) at low speeds (~speed 1-4 depending on your mixer). **The sample should NOT be vortexed, but it should rather be constantly mixed**. If the mixer is fast enough to vortex the sample, decrease the speed accordingly.

   **NOTE:** It is very important to **drop the ice-cold methanol slowly (>30 sec)** in the step below.
7. Place the 15 mL tube on the vortex mixer while it is on the "On" switch, then slowly add 4.5 mL of ice-cold methanol in a **dropwise manner (>30 sec)**.
8. Incubate the cells overnight in the -20°C freezer. **(SAFE STOP - sample can be stored in the -20°C freezer for up to 7 days)**

## B. Blocking (Day 2)

From this point forward, at every centrifugation step, it is crucial to spin down the cells at 850 x g. Decreasing the spin down speed will result in cell loss.

1. Thaw the following reagents at room temperature:
   - Thaw 1 bottle of InTraSeq™ Wash Buffer.
   - Thaw the InTraSeq™ Blocking Buffer B.
   - Thaw and pipette mix the InTraSeq™ Blocking Buffer A.
   - Thaw and vortex mix the InTraSeq™ Reducing Agent until the precipitate disappears.
2. Once thawed, place the reagents on ice.
3. Prepare the heat/cooled InTraSeq™ Blocking Buffer B:
   a. Aliquot 130 µL InTraSeq™ Blocking Buffer B into a sterile 1.5 mL microcentrifuge tube. Return the stock InTraSeq™ Blocking Buffer B to the freezer.
   b. Heat the aliquoted tube at 95°C for 5 min.

# InTraSeq™ 3' Protocol

c. Spin the tube down briefly then place it on ice. Use this heat/cooled InTraSeq™ Blocking Buffer B in the steps below.

4. Prepare the **scBlock** (see table below), pipette mix. Store on ice.

| scBlock | Volume |
|---|---|
| InTraSeq™ Blocking Buffer A | 860 µL |
| Heat/cooled InTraSeq™ Blocking Buffer B | 98 µL |
| InTraSeq™ Reducing Agent | 40 µL |
| InTraSeq™ RNase Inhibitor | 2 µL |
| **TOTAL Volume** | **1,000 µL** |

5. Centrifuge the cells at 850 x g for 5 min at 4°C.

6. Remove and discard the supernatant, then add the **scBlock** to the cells. Pipette mix to resuspend the cells, then keep the cells on ice for at least 30 min.

7. During the **scBlock** incubation, prepare the **Antibody Master Mix** as follows:

   a. Determine the volume of Antibodies **(Vol. of Abs.)** based on the number of InTraSeq™ 3' Antibody Cocktails and Individual InTraSeq™ 3' Conjugates (Ind. InTraSeq™ 3' Conj. or Ind. Conj.) being used (see table below). Limit the total volume to 30 µL.

| | Volume (Cocktails only) | Volume (Ind. Conj. only) | Volume (Cocktails + Ind. Conj.) |
|---|---|---|---|
| InTraSeq™ 3' Cocktail | 5 µL (per cocktail) | N/A | ___ µL (5 µL per cocktail) |
| Ind. InTra-Seq™ 3' Conj. | N/A | 3 µL (per Ind. Conj.) | ___ µL (3 µL per Ind. Conj.) |
| **Vol. of Abs.** | ___ µL | ___ µL | ___ µL |

b. Prepare the **Antibody Master Mix** (see table below) using the **Vol. of Abs.** (max 30 µL) calculated above. Gently pipette mix to minimize bubble formation, then store on ice.

| Antibody Master Mix | Volume (Cocktails only) | Volume (Ind. Conj. only) | Volume (Cocktails + Ind. Conj.) |
|---|---|---|---|
| **Vol. of Abs.** | ___ µL | ___ µL | ___ µL |
| InTraSeq™ Wash Buffer | 30 µL - **Vol. of Abs.** (see row above) | | |
| InTraSeq™ Staining Buffer | 55 µL | 55 µL | 55 µL |
| Heat/cooled InTraSeq™ Blocking Buffer B | 10 µL | 10 µL | 10 µL |
| Rabbit (DA1E) mAb IgG XP® Isotype Control (InTraSeq™ 3' Conjugate 3000) | 3 µL | 3 µL | 3 µL |
| Histone H3 (D1H2) XP® Rabbit mAb (InTraSeq™ 3' Conjugate 3002) | 3 µL | 3 µL | 3 µL |
| InTraSeq™ RNase Inhibitor | 2 µL | 2 µL | 2 µL |
| **TOTAL Volume** | **103 µL** | **103 µL** | **103 µL** |

8. After the 30 min **scBlock** incubation, add 2 mL InTraSeq™ Wash Buffer to the cells and pipette mix.

9. Filter the cells through a sterile 40-micron Falcon cell strainer into a 50 mL tube.

   **NOTE:** To maximize cell recovery, apply gentle pressure to the pipette tip against the strainer while filtering. (See image)

10. Transfer the flow through/filtered cells into a new 15 mL tube and immediately start section **C. Immunostaining (Day 2)**.

    **NOTE:** Store the remaining InTraSeq™ Wash Buffer at 4°C for use on Day 3.

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## C. Immunostaining (Day 2)

From this point forward, whenever removing and discarding the supernatant, it is crucial to maintain the cell pellet submerged (in ~40 µL). Drying the cells will negatively impact the RNA signal and result in cell loss.

1. Centrifuge the cells at 850 x g for 5 min at 4°C.
2. Remove and discard supernatant while retaining ~40 µL in the tube.
3. Add 100 µL **Antibody Master Mix** to the cells. Pipette mix to resuspend the cells.
4. Transfer to a sterile 1.5 mL LoBind microcentrifuge tube and incubate at 4°C overnight (for ~16 hr).

   **NOTES:**

   - Do not incubate the cells for longer than 20 hr.
   - It is crucial to maintain the overnight incubation at 4°C.

## D. Washes and Count (Day 3)

**Before starting:**

Prepare the **Modified InTraSeq™ Wash Buffer**. Gently mix to minimize bubble formation, then keep on ice.

**NOTE:** If there is remaining InTraSeq™ Wash Buffer in the stock bottle, store it at -20°C.

| Modified InTraSeq™ Wash Buffer | Volume |
|---|---|
| InTraSeq™ Wash Buffer | 14 mL |
| InTraSeq™ RNase Inhibitor | 26 µL |

1. Add 1 mL **Modified InTraSeq™ Wash Buffer** to the 1.5 mL microcentrifuge tube containing the cells. Pipette mix then transfer the mixture to a 15 mL tube.
2. Add 2 mL **Modified InTraSeq™ Wash Buffer** so the total volume is 3 mL.
3. Centrifuge at 850 x g for 5 min at 4°C. Remove and discard supernatant while retaining ~40 µL in the tube.
4. Add 1 mL **Modified InTraSeq™ Wash Buffer**. Pipette mix to resuspend the cells, then add another 3 mL **Modified InTraSeq™ Wash Buffer** so the total volume is 4 mL.
5. Repeat steps "3" and "4", then filter the 4 mL cell suspension through a sterile 40-micron Falcon cell strainer into a 50 mL tube. Transfer the flow through/filtered cells into a new 15 mL tube.

   **NOTE:** To maximize cell recovery, apply gentle pressure to the pipette tip against the strainer while filtering. (See image)

6. Centrifuge the cells at 850 x g for 5 min at 4°C. Remove and discard supernatant while retaining ~40 µL in the tube.
7. Add 100 µL **Modified InTraSeq™ Wash Buffer** to the cells. Pipette mix to resuspend the cells, then keep the cells on ice.

   **NOTE:** From this point forward, keep the cells in the **Modified InTraSeq™ Wash Buffer** including any necessary dilutions. **Using any other buffer or solution will result in RNA degradation.**
8. Count the cells. Refer to the Cell Suspension Volume Calculator Table in the 10x Genomics Chromium Single Cell 3' Kits with Feature Barcoding technology protocol to determine the desired cell concentration.

   **IMPORTANT:** Dilute the cells using **ONLY** the **Modified InTraSeq™ Wash Buffer** to reach the desired cell concentration.

   **NOTE:** If cell clumps are observed after counting, additional pipette mixing is recommended before proceeding to the single-cell experiment.
9. Process the cells using the 10x Genomics Chromium Single Cell 3' Kits with Feature Barcoding technology.