



# Custom Antibody Conjugation

Looking for a specific antibody conjugate? Cell Signaling Technology (CST) can conjugate it for you.

- Validated, optimized, and tested for stability to the same standards as the conjugated antibodies available in our catalog.
- Save time and avoid the pitfalls associated with do-it-yourself (DIY) kits.
- Need to validate the conjugated antibody yourself? We also offer basic conjugation services to match your timeline and budget needs.

## Advantages of CST Custom Conjugation:

**Flexible:** Tiered service offerings range from basic antibody conjugation to full validation and stability testing of conjugates.

**Validated:** Conjugates are tested in key applications using biologically relevant cell systems and controls.

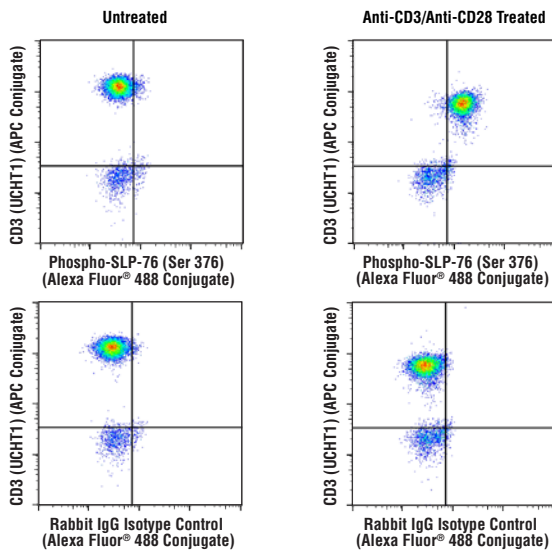
**Optimized:** Flexible conjugation chemistry, free dye and/or antibody purification, and identification of optimal degree of labeling (DOL) are utilized to ensure the best signal-to-noise ratio.

**Support:** Receive consultation and technical support from the conjugation experts who produce our ready-made conjugates.

## Application-Validated Antibody Conjugates

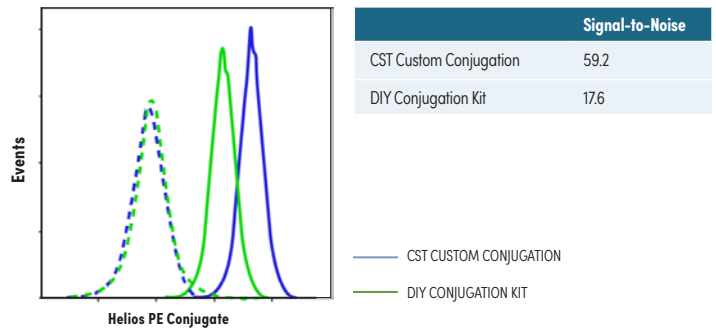
The following data illustrate the performance of Cell Signaling Technology (CST) ready-made conjugated antibodies in different applications. These are representative of the quality and validation of custom conjugates from CST.

### Flow Cytometry



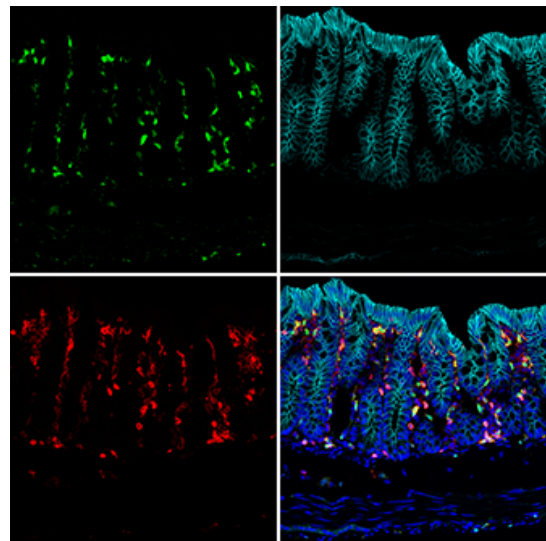
**Phospho-SLP-76 (Ser376) (E3G9U) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) #47876:** Flow cytometric analysis of human PBMCs, untreated (left column) or treated with cross-linked anti-CD3 plus anti-CD28 (right column), using Phospho-SLP-76 (Ser376) (E3G9U) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) (top row) or concentration-matched Rabbit (DAIE) mAb IgG XP<sup>®</sup> Isotype Control (Alexa Fluor<sup>®</sup> 488 Conjugate) #2975 (bottom row). Co-stained with CD3 (UCHT1) Mouse mAb (APC Conjugate) #19881.

## CST Custom Conjugates Deliver Superior Performance



**DIY Conjugation Kit compared to CST Custom Conjugation:** Comparison of PE conjugation of Helios (D8W4X) XP<sup>®</sup> Rabbit mAb by flow cytometry, using CST custom conjugation method (blue) or another company's DIY conjugation kit (green), on both RL-7 (dashed lines), a low-expressing cell line, and Jurkat (solid lines), a high-expressing cell line. A comparison of the calculated signal-to-noise ratio is also shown. Avoid the inefficiencies caused by DIY conjugation such as suboptimal labeling, interference with specificity, and/or destabilization/degradation that can negatively affect antibody performance and decrease yields.

### Immunofluorescence



**TIPE2 (E5Q2S) Rabbit mAb #53842:** Confocal IF analysis of mouse colon using TIPE2 (E5Q2S) Rabbit mAb (green). After blocking free secondary antibody binding sites with Rabbit (DAIE) mAb IgG XP<sup>®</sup> Isotype Control #3900, the tissues were then labeled using Non-phospho (Active)  $\beta$ -Catenin (Ser45) (D2U8Y) XP<sup>®</sup> Rabbit mAb (Alexa Fluor<sup>®</sup> 488 Conjugate) #70034 (cyan pseudo-color) and CD45 (D3F8Q) Rabbit mAb (Alexa Fluor<sup>®</sup> 647 Conjugate) #81143 (red). Samples were mounted in ProLong<sup>®</sup> Gold Antifade Reagent with DAPI #8961 (blue).

For Research Use Only. Not For Use In Diagnostic Procedures.

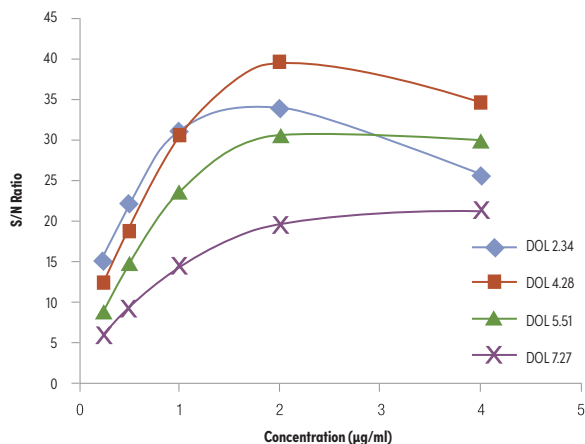
[www.cst-science.com/customconjugation](http://www.cst-science.com/customconjugation)



Cell Signaling  
TECHNOLOGY<sup>®</sup>

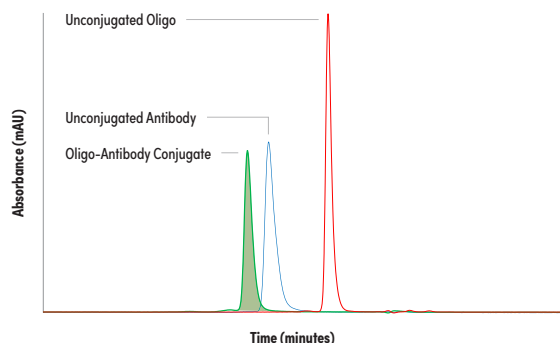
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## Degree of Labeling (DOL) to Ensure Specific Signal Intensity



**DOL service option for fluorophores:** Conjugation to certain fluorescent dyes (Alexa Fluor® and Pacific Blue™) is optimized by degree of labeling (DOL) testing to identify the optimal antibody:dye molecular ratio, resulting in conjugates with maximum specific signal intensity.

## Size Exclusion to Isolate Oligo-antibody Conjugate



**Final Conjugate Isolated from Unconjugated starting materials:** Size Exclusion Chromatogram comparing purified Oligo-Antibody Conjugate (green peak) to unconjugated Antibody (blue line) and unconjugated Oligo (red line). CST uses Size exclusion chromatography to separate out free materials and deliver only the purified Oligo-Antibody conjugate product to the customer, ensuring a higher quality reagent.

## Conjugation Services Available:

Conjugation Type	Service Tier	Service Includes
<b>Fluorophores Type I:</b> Alexa Fluor®; Cyanine (CyDye®) and Pacific Blue™ dyes	Basic	Conjugation + removal of free dye
	Tier I	Degree of labeling (DOL) + Basic Service
	Tier II	Validation <sup>1</sup> + Tier I Service
	Tier III	Stability testing + Tier II Service
<b>Fluorophores Type II:</b> Phycoerythrin (PE), PE-CyDye® tandems (e.g. PE-Cy®7), and allophycocyanin (APC)	Basic	Conjugation + purification
	Tier I	Validation <sup>1</sup> + Basic Service
	Tier II	Stability testing + Tier I Service
<b>Haptens:</b> Biotin, Digoxigenin (DIG), DNP, and Dansyl	Basic	Conjugation + removal of free biotin
	Tier I	Validation <sup>2</sup> + Basic Service
<b>Beads:</b> Sepharose®, Magnetic, Agarose	Basic	Conjugation + removal of free antibody
	Tier I	Validation <sup>3</sup> + Basic Service
<b>Enzymes:</b> Horseradish peroxidase (HRP)	Basic	Conjugation + purification
	Tier I	Validation <sup>2</sup> + Basic Service
<b>Oligonucleotides</b> <sup>4</sup>	Basic	Conjugation + purification
	Tier I	Biophysical Characterization + Basic Service

1. Standard validation application method is Flow Cytometry.

2. Standard validation application method is Western Blot.

3. Standard validation application method is IP.

4. Oligonucleotide conjugation offering may be limited for certain IP protected technologies.

**Interested in a custom conjugation or a specific conjugation type not listed above?**

**Contact us:** [cst-science.com/conjugationrequest](http://cst-science.com/conjugationrequest)

## Oligonucleotide Conjugation Services

- Biophysical characterization checks integrity, size, and confirms no aggregation, free antibody or free oligo, and is typically performed by analytical chromatogram.
- Basic Certificate of Analysis (COA) includes Oligo sequence, antibody concentration, and DOL information. Tier I COA also includes biophysical characterization results.
- DOL offering allows one to analyze individual fractions or multifraction pools to select the best product.

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