Achieving rapid antibody labeling with Capturem technology

Introduction

Labeling antibodies (Abs) is one key way researchers expand their utility for many applications. The addition of fluorescence or biotin enables antibodies to be used for a variety of immunochemistry-based techniques, while antibody-drug conjugates are a rapidly growing area of pharmaceutical research. However, traditional conjugation methods require purified starting material and long incubation times. To improve this process, we've adapted Capturem purification technology to enable rapid on-column conjugations that can be performed directly on unpurified starting material, resulting in labeled antibodies in less than 15 minutes.

In the examples below, we demonstrate the labeling of a mouse IgG targeting Cas9 with fluorescein and biotin. The labeled antibody recognizes its antigen, indicating that the epitope is not negatively affected by the labeling process. **To view the conjugation protocols and learn how you can perform rapid antibody labeling, fill out the form on the left. On mobile devices the form is in the menu button.**

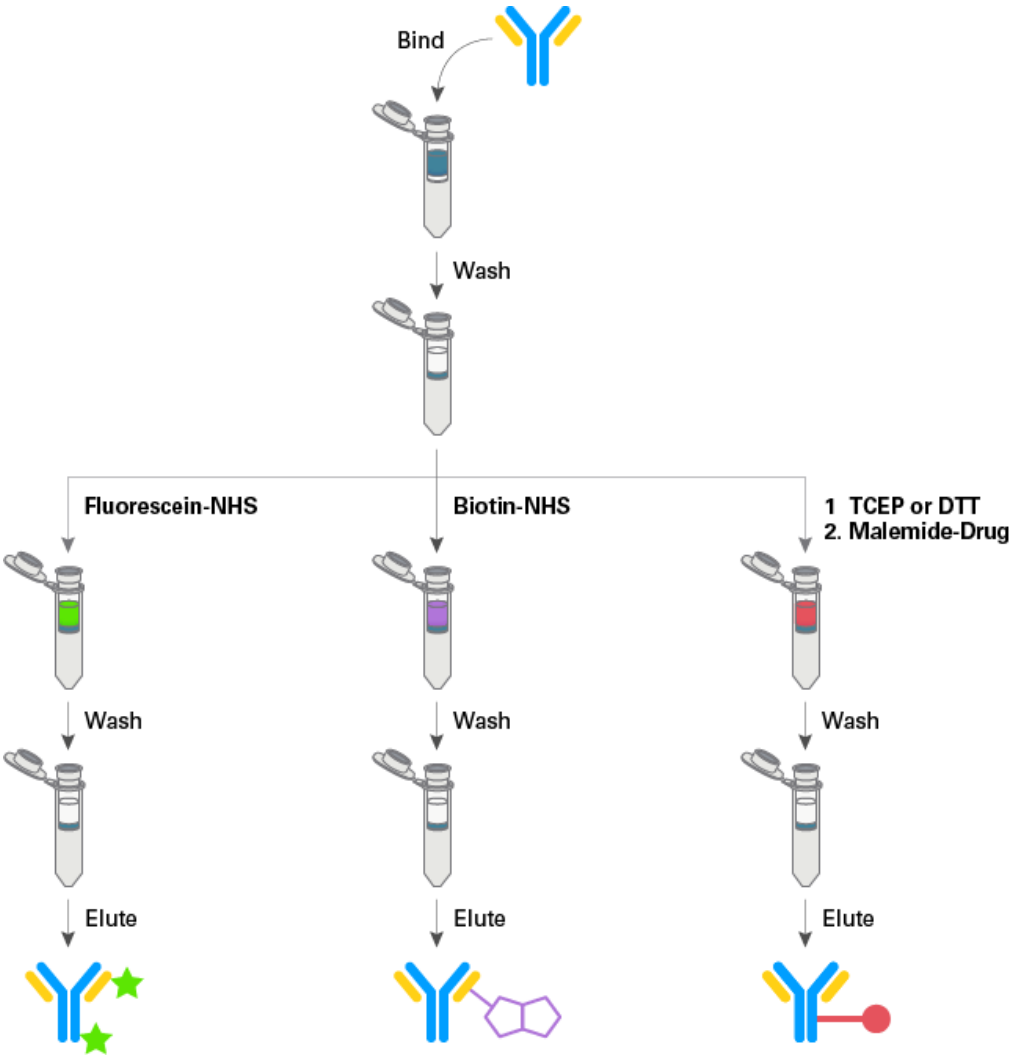

Figure 1. Schematic demonstrating a workflow for rapidly labeling antibodies directly from any starting solution. First, the antibody is diluted in binding buffer and the column is equilibrated with binding buffer. Next, the diluted antibody is loaded onto the column and washed with a volume of wash buffer. Your labeling reagent is then dissolved in the appropriate buffer and spun through the column to complete the antibody labeling process. To retrieve the labeled antibody, one more wash is performed and then the antibody is eluted with an appropriate elution buffer. The entire labeling process can be completed in 15

minutes.

Fluorescein labeling

The first demonstration of rapid antibody labeling was done using a fluorescein-NHS conjugate. Our starting material was hybridoma medium containing a mouse IgG targeting Cas9. We loaded 150 µg of antibody onto the column following our protocol (available by filling out the form). After flowing through the fluorescein-NHS, we washed and then eluted in two steps, resulting in the capture of 40 µg of fluorescein-labeled antibody.

We measured the molar ratio of fluorescein:Ab using A280 and A493 measurements. By varying the input amount of fluorescein-NHS (12 eq., 20 eq., 50 eq., or 100 eq.; where eq. stands for molar equivalents), we varied the number of fluorescent molecules per antibody from 2.97–3.3. While increasing the amount of NHS-fluorescein in the reaction did slightly increase the ratio of label to antibody, the best performance was achieved at 12 and 20 eq. Higher levels of fluorescein (50 and 100 eq.) resulted in an excess of free dye in the eluted samples (Figure 2).

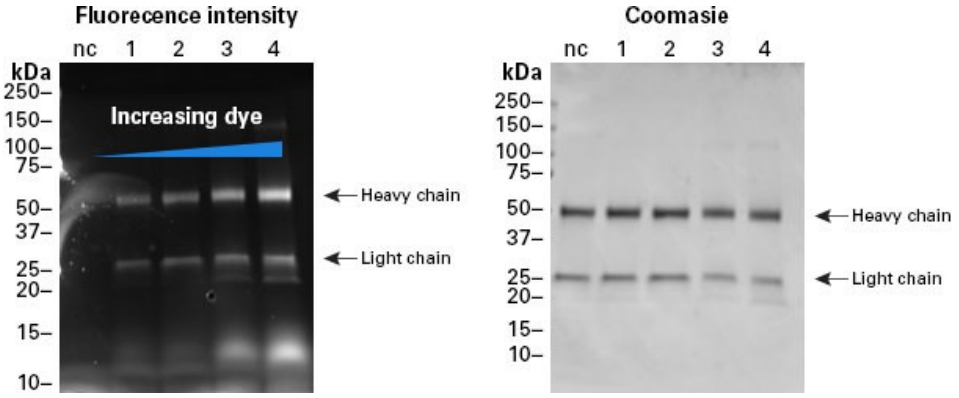

Figure 2. Increasing fluorescein concentration in the labeling reaction results in higher ratios of fluorescein:Ab. The left gel image shows the fluorescence signal per well and demonstrates that the fluorescence signal rises with the ratio of dye molecules per antibody in the labeling reaction. Each well has an equal mass of antibody, as seen in the Coomassie-stained gel on the right. nc: Negative control. Lane 1: 12-eq. reaction. Lane 2: 20-eq. reaction. Lane 3: 50-eq. reaction. Lane 4: 100-eq. reaction.

Antibody biotinylation

Another common labeling reaction involves attaching biotin to antibodies for use in a variety of assay formats, including using primary-secondary labeling with fluorescence for FACS and imaging or setting up ELISA experiments. We followed a similar protocol to the fluorescein labeling experiments but with biotin-NHS as the labeling reagent for the mouse IgG targeting Cas9 (Figure 3).

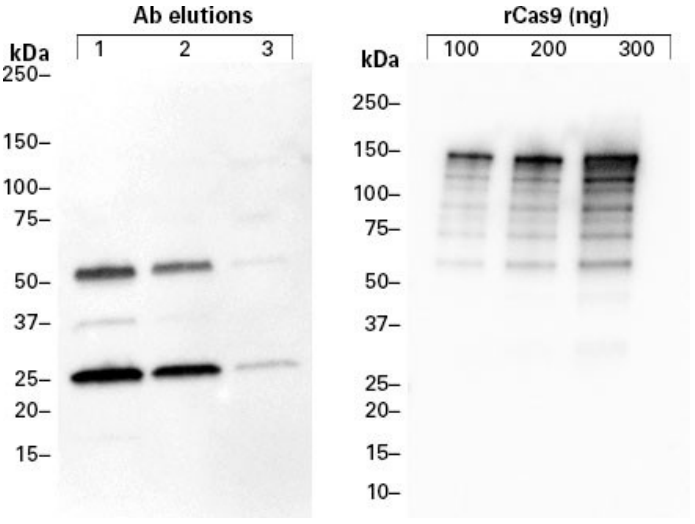

Figure 3. Western blot of Ab elutions off the column (left) and Western blot detection of recombinant Cas9 (rCas9) using the eluted biotinylated Ab

(right). The elutions demonstrate that >90% of the biotinylated Ab is captured in the first two elutions. In the Western blot on the right, we loaded between 100–300 ng of Cas9 in each lane and then used the biotinylated anti-Cas9 Ab to detect Cas9, demonstrating that the binding epitope remains active after labeling and eluting off the Capturem Protein G Miniprep Columns.

Labeling protocol

A detailed protocol is available by filling in the form in the lefthand navigation column. On mobile devices the form is in the menu button.

All labeling reactions were performed on Capturem Protein G Miniprep Columns. EZ-Link Sulfo-NHS-LC-Biotin (Cat. No. 21335) and fluorescein-NHS (Cat. No. 46410) were purchased from Thermo Fisher Scientific. Biotin-NHS was manufactured in-house. Anti-Cas9 Ab is available from Takara Bio USA (Cat. No. 632628).

Related Products

Cat. #	Product	Size	License	Quantity	Details
635725	Capturem™ Protein G Miniprep Columns	10 Columns		*	

Capturem Protein G Miniprep consists of single-use disposable columns for simple, rapid purification of antibodies that yield 50–100 µg of purified antibody per column, depending upon the sample type, species, and antibody isotype. These miniprep columns are suitable for purification of antibodies from animal sera, ascites fluid, cell culture media, and other sources.

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