

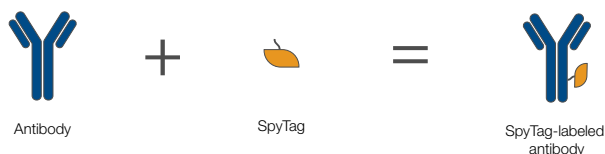
TrailBlazer™ Tag Kit

Instructions for Use

Introduction

The TrailBlazer Tag Kit is the first part of a two-kit antibody labeling system. When used with a TrailBlazer StarBright™ Dye Label Kit, these kits enable the attachment of purified antibodies to a wide range of StarBright Dyes utilizing innovative SpyTag and SpyCatcher technology — often termed molecular superglue. First, SpyTag is attached to your antibody using the TrailBlazer Tag Kit (Figure 1A). Next, this antibody is mixed with StarBright Dye–labeled SpyCatcher using the TrailBlazer StarBright Dye Label Kit (Figure 1B). A spontaneous reaction between SpyTag and SpyCatcher results in an irreversible covalent isopeptide bond, creating a labeled antibody.

A. TrailBlazer Tag Kit.



B. TrailBlazer StarBright Dye Label Kit.

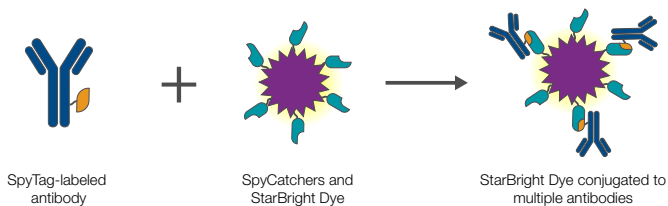


Fig. 1. Overview of TrailBlazer Tag and TrailBlazer StarBright Dye Label Kits. **A**, attachment of a SpyTag to a purified antibody. **B**, attachment of a StarBright Dye to the SpyTag-labeled antibody.

The TrailBlazer Tag and TrailBlazer StarBright Dye Label Kits are compatible with most purified antibodies. Visit bio-rad-antibodies.com/Trailblazer-Kit for further information. TrailBlazer Kits are optimized to label 0.1 mg of purified antibody at a concentration greater than 0.2 mg/ml and are compatible with all IgG isotypes. IgM antibodies have been successfully labeled, but the effectiveness of labeling with this isotype may be variable and antibody-dependent. TrailBlazer Kits are compatible with purified antibodies in various buffers and stabilizing proteins, including 0.5% BSA and 0.09% sodium azide.

These instructions are for the TrailBlazer Tag Kit only. Visit bio-rad-antibodies.com/Label-SB-Kit-IFU for TrailBlazer StarBright Dye Label Kit instructions.

Size

The kit is designed to label 0.1 mg of purified antibody and is available in two different pack sizes: 1 x 0.1 mg and 3 x 0.1 mg.

Storage

Store all kit components at 4°C.

Stability

The kit is stable for one year from the date of receipt under recommended storage conditions.

Kit Components

Kit Component	Quantity for 1 x 0.1 mg	Quantity for 3 x 0.1 mg
Conjugation Buffer	1 vial (5 ml)	3 vials (5 ml)
Reagent A	1 vial (powder)	3 vials (powder)
Tag	1 vial (lyophilized)	3 vials (lyophilized)
Filter in collection tube	1 ea	3 ea
Desalting column	2 ea	6 ea

Required Equipment and Reagents

- Ultrapure water or equivalent
- 1.5 ml microcentrifuge tubes
- Microcentrifuge; minimum speed of 5,000 x g
- Vortex mixer
- 2–20 µl pipet and tips
- 20–200 µl pipet and tips
- 200–1,000 µl pipet and tips

Protocol

1. Antibody preparation

- 1.1 Add 100 μg of purified antibody to the filter and then place the filter into the collection tube (Figure 2). Note: The filter has graduations to indicate volume.
- 1.2 Add Conjugation Buffer to reach a total volume of 500 μl . Cap the tube and invert to mix.
- 1.3 Centrifuge at 5,000 \times g until the volume reaches \sim 50 μl (approximately 5 minutes). Discard the eluate in the collection tube. Retain the collection tube for steps 1.4 and 1.5.
Wash 1
- 1.4 Repeat steps 1.2–1.3 two additional times.
Wash 2 wash 3
- 1.5 Ensure the antibody volume is \sim 50 μl and no more than 100 μl . If the volume of antibody exceeds 100 μl , perform further centrifugations (5,000 \times g for 2 min) until the volume is less than 100 μl . Invert the filter and place into the empty collection tube. (See Figure 2.)

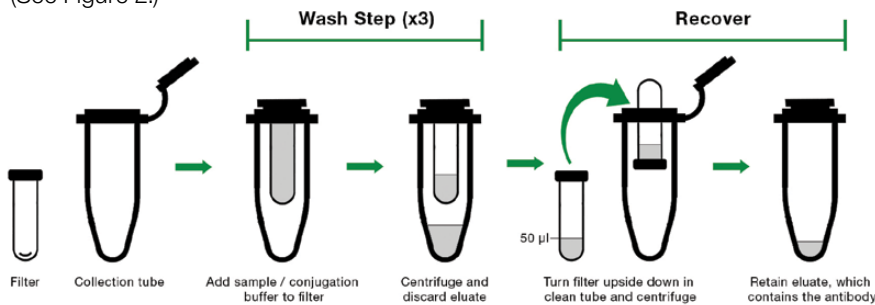


Fig. 2. Filter components and usage overview.

- 1.6 Centrifuge the collection tube with the inverted filter for 30 sec at 5,000 \times g to recover the antibody in the tube. Retain the eluate containing the antibody and discard the filter.
- ### 2. Antibody modification
- 2.1 Prepare Reagent A by adding 75 μl of Conjugation Buffer. Pulse spin and vortex to dissolve. Pulse spin again to collect the volume at the bottom of the tube.
 - 2.2 Add 2 μl of prepared Reagent A to the antibody solution. Briefly vortex to mix and pulse spin. Discard excess prepared Reagent A.
 - 2.3 Incubate at room temperature for 60 min.
Incubation start time: _____ Incubation end time: _____
 - 2.4 While incubating, prepare a desalting column. Note: Columns may be prepared up to 1 hour before use.
 - 2.4.1 Snap open the bottom of the desalting column and insert the column into a 2 ml collection tube. Remove and discard the column cap and centrifuge for 2 min at 1,000 \times g. Discard the eluate.
 - 2.4.2 Apply 500 μl of Conjugation Buffer and centrifuge for 1 min at 1,000 \times g. Discard the eluate.
Wash 1
 - 2.4.3 Repeat step 2.4.2 two additional times. For the third wash, add Conjugation Buffer but wait to centrifuge until immediately before proceeding to step 2.5. After the third wash, ensure there is no remaining solution in the column. If necessary, briefly centrifuge until no solution remains.
Wash 2 wash 3
 - 2.5 Insert the prepared desalting column into a fresh 1.5 ml microcentrifuge tube and apply the entire sample from step 2.3 to the column.
 - 2.6 Centrifuge at 1,000 \times g for 4 min and retain the eluate containing the modified antibody. Discard the desalting column.

3. Attachment of SpyTag to the antibody

- 3.1 Prepare the Tag by adding 5 µl of ultrapure water, then pulse spin and vortex briefly to mix. Pulse spin again to collect the volume at the bottom of the tube.
- 3.2 Add 2 µl of prepared Tag to the prepared antibody. Vortex briefly and pulse spin. Discard any excess Tag.
- 3.3 Incubate at room temperature for 30 min. Note: Incubation may be extended to 3 hours if convenient.
Incubation start time: _____ Incubation end time: _____
- 3.4 While incubating, prepare a second desalting column. Note: Columns may be prepared up to 1 hour before use.
 - 3.4.1 Snap open the bottom of a new desalting column and insert the column into a 2 ml collection tube. Remove and discard the column cap and centrifuge for 2 min at 1,000 x g. Discard the eluate.
 - 3.4.2 Apply 500 µl of Conjugation Buffer and centrifuge for 1 min at 1,000 x g. Discard the eluate.
Wash 1
 - 3.4.3 Repeat step 2.4.2 two additional times. For the third wash, add Conjugation Buffer but wait to centrifuge until immediately before proceeding to step 3.5. After the third wash, ensure there is no remaining solution in the column. If necessary, briefly centrifuge until no solution remains.
Wash 2 wash 3
- 3.5 Insert the prepared desalting column into a fresh 1.5 ml microcentrifuge tube and transfer the entire sample from step 3.3 to the column.
- 3.6 Centrifuge at 1,000 x g for 4 min and retain the eluate containing the modified antibody. Discard the desalting column.

This completes the TrailBlazer Tag Kit protocol. Proceed with the TrailBlazer StarBright Dye Label Kit or store the antibody with the SpyTag at 4°C for up to 2 weeks.

TrailBlazer Tag and StarBright Dye Label Protocol Worksheet

Visit bio-rad-antibodies.com/TrailBlazer-Kit-Worksheet for a convenient protocol worksheet, available for download. This worksheet allows you to record and store each step of the tag and label process for later reference.

TrailBlazer Tag and TrailBlazer StarBright Dye Label Kit Range

Visit bio-rad-antibodies.com/TrailBlazer-Label-Kit-Range for the full range of available TrailBlazer Tag and StarBright Dye Label Kits.

Further Information about StarBright Dyes

Visit bio-rad-antibodies.com/StarBright to select the most appropriate StarBright Dye for your application, including details on excitation and emission wavelengths.

Visit bio-rad-antibodies.com/TrailBlazer-Kit for more information.

Contact the technical services team at bio-rad.com/Contact-Us for further assistance.

For Research Use Only.



Visit bio-rad-antibodies.com/TrailBlazer-Kit for more information.

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Products containing SpyTag1, SpyTag3, SpyCatcher1, or SpyCatcher3 and/or their use are covered by the following U.S. patents and/or pending U.S. patent application or their foreign counterparts owned by or under license to Bio-Rad Laboratories, Inc., including, but not limited to, U.S. Patent Nos. 9,547,003, 10,247,727, and 10,527,609. Products containing SpyTag2 or SpyCatcher2 and/or their use are covered by the following U.S. patents and/or pending U.S. patent application or their foreign counterparts owned by or under license to Bio-Rad Laboratories, Inc., including, but not limited to, U.S. Patent Nos. 9,547,003, 10,247,727, 10,527,609, and 11,059,867.



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