TrailBlazer™ StarBright™ Dye Label Kit

Instructions for Use

Introduction

The TrailBlazer StarBright Dye Label Kit is the second part of a two-kit antibody labeling system. When used with the TrailBlazer Tag 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, a SpyTag is attached to your antibody using the TrailBlazer Tag Kit (Figure 1A). Next, this antibody is mixed with a 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.

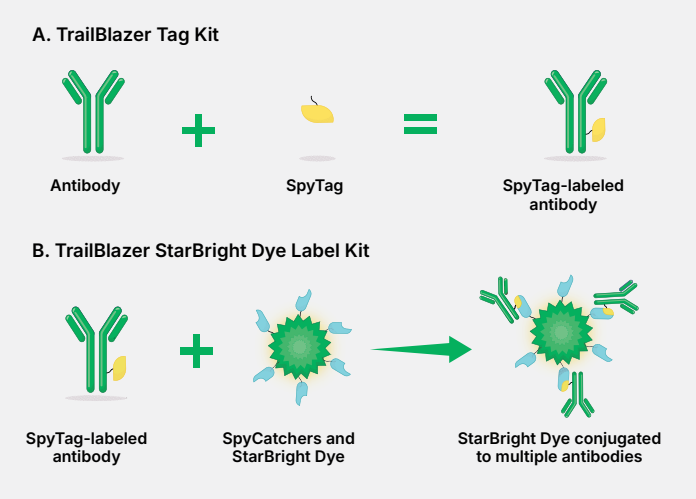


Fig. 1. Overview of TrailBlazer Tag and TrailBlazer StarBright Dye Label Kits. **A**, attachment of a SpyTag to a purified antibody; **B**, attachment of a StarBright Dye to the SpyTag-labeled antibody.

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

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
StarBright Dye–Catcher	1 vial (200 µL)	3 vials (200 µL)
Quenching Reagent	1 vial (lyophilized)	3 vials (lyophilized)
Storage Buffer	1 vial (100 µL)	3 vials (100 µL)
Spin Column	1 ea	3 ea
2 ml amber collection tube	1 ea	3 ea

Required Equipment and Reagents

- Ultrapure water or equivalent
- 2–20 µL pipet and tips
- 20–200 µL pipet and tips
- 200–1,000 µL pipet and tips
- Microcentrifuge; minimum speed of 1,500 x g
- Vortex mixer
- End-over-end rotating mixer (see Figure 2)

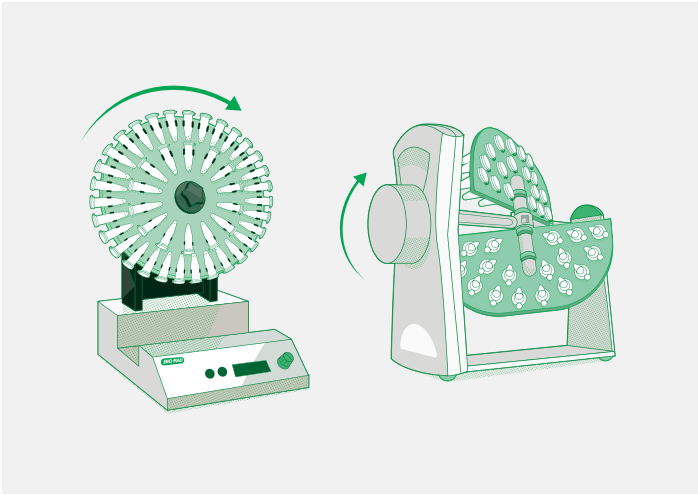


Fig. 2. Examples of end-over-end rotating mixers.

Protocol

1. Add the entire volume of the antibody SpyTag product from the TrailBlazer Tag Kit to the vial containing 200 µL of StarBright Dye–Catcher. Vortex briefly to mix and pulse spin. Incubate at room temperature for 30 min. **Optional:** Incubation may be extended to overnight at 4ºC if convenient.

   Incubation start time: _____ Incubation end time: _____

2. Transfer the entire sample to the Spin Column, keeping the bottom of the column intact until step 3. Cap the column and briefly vortex to mix. Continue mixing on a rotary mixer at room temperature for 60 min. Ensure the slurry is mixing properly and has not settled at the bottom of the tube. If it settles, briefly vortex to resuspend and continue mixing on an end-over-end rotating mixer.

   Incubation start time: _____ Incubation end time: _____

3. Break open the bottom of the Spin Column and insert the column into the 2 mL amber collection tube. Slightly loosen the cap of the Spin Column by turning the cap one-quarter of a turn. Place the tubes into a microcentrifuge and centrifuge at 1,500 x g for 2 min to clarify the purified conjugate. Retain the eluate containing the labeled antibody and discard the Spin Column.
4. Prepare Quenching Reagent by adding 5 µL of ultrapure water. Pulse spin and vortex briefly to mix. Pulse spin again to collect the volume at the bottom of the tube.
5. Add 4 µL of prepared Quenching Reagent and 45 µL of Storage Buffer to the labeled antibody. Vortex briefly to mix. Discard excess Quenching Reagent and Storage Buffer.

This completes the TrailBlazer Label Kit protocol. Proceed with your StarBright Dye–labeled antibody in downstream applications or store at 4ºC in the dark for up to 12 months.

Performing a titration is recommended to determine the optimal amount for each antibody and experimental condition. For flow cytometry, a typical starting point is 5 µL of labeled antibody to stain 10^6 cells, while the optimal dilution for western blotting typically ranges from 1:1,000–1:5,000.

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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** the full range of available TrailBlazer Tag and TrailBlazer 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.

StarBright Dyes

TrailBlazer

Visit **bio-rad-antibodies.com** 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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