

StarBright™ Blue Fluorescent Secondary Antibodies

Instruction Manual

Catalog #	Description
12005866	Goat Anti-Mouse IgG StarBright Blue 520, 400 µl
12005869	Goat Anti-Rabbit IgG StarBright Blue 520, 400 µl
12004158	Goat Anti-Mouse IgG StarBright Blue 700, 400 µl
12004161	Goat Anti-Rabbit IgG StarBright Blue 700, 400 µl

Trial size (80 µl) available. See back page for ordering information.

Shelf life: 1 year at -20°C lyophilized; 6 months at 4°C after resuspension.

Visit bio-rad.com/StarBright for more detailed information about this product.

For technical support call your local Bio-Rad office. In the U.S. call 1-800-4BIORAD (1-800-424-6723).

BIO-RAD

Introduction

Bio-Rad's StarBright Blue Secondary Antibodies are fluorescently tagged detection reagents for western blotting. These tagged antibodies are exceptionally bright and resistant to photobleaching, and they offer unparalleled sensitivity and reproducibility in western blotting.

Western blots visualized with StarBright Blue 520 (excitation: 440 nm, emission: 520 nm) and StarBright Blue 700 Antibodies (excitation: 470 nm, emission: 700 nm) may be imaged using the fluorescence-enabled ChemiDoc™ MP Imaging System.

StarBright Blue 520 Secondary Antibodies may be used together with StarBright Blue 700 Secondary Antibodies and other traditional fluorescent antibodies for multiplexing in addition to the hFAB™ Rhodamine Anti-Housekeeping Protein Primary Antibodies for normalizing protein loading amounts.

Instructions for Use

Preparation

Resuspend contents of the tube in the indicated volume of distilled or deionized water and leave on ice for at least 30 min prior to use. The resuspended solution may be stored at 4°C in the dark for up to 6 months. **Do not freeze the solubilized material.**

Brief centrifugation (pulse spin for 2–3 sec at maximum speed in a tabletop microcentrifuge) may be used to collect the contents at the bottom of the tube. Do not centrifuge more than 10 sec.

General Guidelines

StarBright Blue Secondary Antibodies may be used for detection on low fluorescence polyvinylidene difluoride (LF PVDF) or nitrocellulose membranes.

For blocking and washing, use 15 ml for a mini gel. For primary and secondary antibody incubations, use 10 ml for a mini gel. Use the smallest flat bottom tray that will accommodate the blot.

Protect the blot from light (for example, using aluminum foil) during incubations with fluorescent antibodies. Image immediately after step 5 for best results.

Do not allow the blot to dry out during washes or antibody incubations.

We recommend using Precision Plus Protein™ All Blue Standards (2–3 µl) since red- or pink-colored standards may fluoresce brightly in the rhodamine imaging channel and can interfere with data acquisition. Shake or rock well (without spilling) during incubations with the StarBright Blue Secondary Antibodies.

Dilute StarBright Blue Secondary Antibodies into incubation solution immediately prior to use.

StarBright Blue Secondary Antibodies may be used successfully in many different immunodetection protocols. The following protocol is recommended for detection with high sensitivity, low background, and minimal nonspecific cross-reactivity.

Protocol

1. **Block:** 1 hr at room temperature (RT) with Tris buffered saline (TBS) + 1% casein (catalog #1610782).
2. **Incubate in primary antibody:** Dilute and incubate the primary antibody as specified in your protocol or by the vendor. If no protocol is provided with the primary antibody, it may be diluted in TBS + 1% casein buffer.
3. **Wash:** 5 x 5 min at RT with TBST (TBS + 0.05% Tween 20).
4. **Incubate with StarBright Blue Secondary Antibody:** Dilute secondary antibody into TBS + 1% casein + 0.02% SDS for use with LF PVDF membrane. For nitrocellulose membrane blots, dilute the StarBright Blue Antibody in TBS + 1% casein only, omit the SDS.

The presence of 0.02% SDS in the StarBright Incubation Buffer for LF PVDF blots does not affect the performance of other fluorescent antibodies such as DyLight 800 Antibodies or hFAB Rhodamine Anti-Housekeeping Protein Primary Antibodies.

5. **Wash:** 6 x 5 min at RT with TBST.
6. **Image:** Use the ChemiDoc™ MP Imaging System with Image Lab™ Touch Software. Select **StarBright B520** or **StarBright B700** under **Application > Blots** (for Image Lab Touch Software, version 2.3 and later software releases).

If using an hFAB Rhodamine Anti-Housekeeping Protein Antibody for protein loading normalization, configure a multichannel imaging protocol by selecting **Rhodamine** under **Application > Blots** (for Image Lab Touch Software, version 2.3 and later software releases).

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Ordering Information

Catalog # Description

StarBright Blue 520 Secondary Antibodies

- 12005866 **Goat Anti-Mouse IgG StarBright Blue 520**, 400 μ l
- 12005867 **Goat Anti-Mouse IgG StarBright Blue 520**, 80 μ l
- 12005869 **Goat Anti-Rabbit IgG StarBright Blue 520**, 400 μ l
- 12005870 **Goat Anti-Rabbit IgG StarBright Blue 520**, 80 μ l

StarBright Blue 700 Secondary Antibodies

- 12004158 **Goat Anti-Mouse IgG StarBright Blue 700**, 400 μ l
- 12004159 **Goat Anti-Mouse IgG StarBright Blue 700**, 80 μ l
- 12004161 **Goat Anti-Rabbit IgG StarBright Blue 700**, 400 μ l
- 12004162 **Goat Anti-Rabbit IgG StarBright Blue 700**, 80 μ l

Related Products

- 12004163 **Anti-Actin hFAB Rhodamine Antibody**, 200 μ l
- 12004164 **Anti-Actin hFAB Rhodamine Antibody**, 40 μ l
- 12004165 **Anti-Tubulin hFAB Rhodamine Antibody**, 200 μ l
- 12004166 **Anti-Tubulin hFAB Rhodamine Antibody**, 40 μ l
- 12004167 **Anti-GAPDH hFAB Rhodamine Antibody**, 200 μ l
- 12004168 **Anti-GAPDH hFAB Rhodamine Antibody**, 40 μ l
- 1610782 **1x Tris Buffered Saline (TBS) with 1% Casein**, 1 L
- 1706435 **10x Tris Buffered Saline (TBS)**, 1 L
- 1610781 **10% Tween 20**, 1 L
- 1610416 **10% SDS Solution**, 250 ml
- 1610373 **Precision Plus Protein All Blue Standards**, 500 μ l

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