StarBright Blue Fluorescent Secondary Antibodies

Instruction Manual

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

Ordering Information

Catalog #	Description
StarBright Blue 520 Secondary Antibodies	
12005866	**StarBright Blue 520 Goat Anti-Mouse IgG**, 400 µl
12005867	**StarBright Blue 520 Goat Anti-Mouse IgG**, 80 µl
12005869	**StarBright Blue 520 Goat Anti-Rabbit IgG**, 400 µl
12005870	**StarBright Blue 520 Goat Anti-Rabbit IgG**, 80 µl
StarBright Blue 700 Secondary Antibodies	
12004158	**StarBright Blue 700 Goat Anti-Mouse IgG**, 400 µl
12004159	**StarBright Blue 700 Goat Anti-Mouse IgG**, 80 µl
12004161	**StarBright Blue 700 Goat Anti-Rabbit IgG**, 400 µl
12004162	**StarBright Blue 700 Goat Anti-Rabbit IgG**, 80 µl
Related Products	
12004163	**hFAB Rhodamine Anti-Actin Primary Antibody**, 200 µl
12004164	**hFAB Rhodamine Anti-Actin Primary Antibody**, 40 µl
12004165	**hFAB Rhodamine Anti-Tubulin Primary Antibody**, 200 µl
12004166	**hFAB Rhodamine Anti-Tubulin Primary Antibody**, 40 µl
12004167	**hFAB Rhodamine Anti-GAPDH Primary Antibody**, 200 µl
12004168	**hFAB Rhodamine Anti-GAPDH Primary Antibody**, 40 µl
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
12010020	**EveryBlot Blocking Buffer**, 500 ml
12010947	**EveryBlot Blocking Buffer**, 50 ml
1610373	**Precision Plus Protein All Blue Standards**, 500 µl

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10000079534 Ver C (12005871) 19-0857 1219

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 with each other and with other traditional fluorescent antibodies for multiplexing. They may also be used with 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 4ºC 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 blot. For primary and secondary antibody incubations, use 10 ml for a mini blot. 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 4 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.

Blots detected with StarBright Antibodies cannot be stripped because of the high binding of StarBright polymeric nanoparticles to membranes.

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 1x Tris Buffered Saline (TBS) with 1% Casein (catalog #1610782). If using EveryBlot Blocking Buffer, block for 5 min.
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 or EveryBlot Blocking 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 1:2,500 in TBS + 1% casein or EveryBlot Blocking Buffer.

   If using LF PVDF membrane, also add 0.02% sodium dodecyl sulfate (SDS). For nitrocellulose, do not add SDS.

   Note: 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).