

hFAB™ Rhodamine Housekeeping Antibodies

| Catalog # | Description |
|-----------|---|
| 12004163 | Anti-Actin hFAB Rhodamine Antibody, 200 µl |
| 12004164 | Anti-Actin hFAB Rhodamine Antibody, 40 µl |
| 12004165 | Anti-Tubulin hFAB Rhodamine Antibody, 200 µl |
| 12004166 | Anti-Tubulin hFAB Rhodamine Antibody, 40 µl |
| 12004167 | Anti-GAPDH hFAB Rhodamine Antibody, 200 µl |
| 12004168 | Anti-GAPDH hFAB Rhodamine Antibody, 40 µl |

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

Instruction Manual

Visit bio-rad.com/web/HKP 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 hFAB™ Rhodamine Antibodies are fluorescently labeled primary antibodies raised against the housekeeping proteins (HKPs) actin, tubulin, and GAPDH. They are intended to be used for normalizing protein loading in western blotting experiments.

The hFAB Rhodamine Antibodies are themselves sufficient and do not need any secondary antibody for detecting the housekeeping protein. For fluorescence multiplexing, these hFAB Rhodamine Antibodies can be incubated along with a variety of fluorescent secondary antibodies, including our new StarBright™ Blue 700 Secondary Antibodies. hFAB Antibodies are recombinant antibody fragments that are not recognized by conventional secondary antibodies, which means they can be used with primary antibodies of any species against your target protein.

hFAB Antibodies are designed to give a linear response to HKPs in a range typical for their presence in cellular lysates. They are labeled with a rhodamine derivative that has minimal spectral cross talk with StarBright Blue 700 Secondary Antibodies. The fluorophore is excited at around 530 nm (green light) and emits maximally around 580 nm.

Note: hFAB Housekeeping Antibodies were created using human GAPDH, actin, and tubulin sequences but will recognize their respective targets from other mammalian species. The sensitivity may not be as high for non-human samples.

Instructions for Use

Preparation

Resuspend the 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 resuspended solution.**

Brief centrifugation (pulse spin for 2–3 sec at maximum speed on a tabletop microcentrifuge) may be used to collect the contents at the bottom of the tube.

General Guidelines

For optimal results, we recommend using low fluorescence PVDF membranes for transfer.

For blocking and washing, use 2 ml of solution per 10 cm² of membrane (15 ml for a mini gel). For primary and secondary antibody incubations, use 1 ml of solution per 10 cm² of membrane (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 at any time prior to imaging. The blot must be kept moist during imaging for best results. We recommend using all blue protein standards 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.

hFAB Rhodamine Antibodies may be used successfully with StarBright Blue 700 Secondary Antibodies (and other fluorescent secondary antibodies, preferably with emissions >650 nm) 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.
2. **Incubate in primary antibody for target protein:**
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 hFAB Rhodamine Antibody:**
Dilute both reagents into TBS + 1% casein. The recommended starting dilution for hFAB Rhodamine Antibody is 1:1,000 (range 1:1,000–1:10,000). If detecting other target proteins using the StarBright Blue 700 Secondary Antibody, the recommended starting dilution is 1:2,500 (range 1:2,500–1:5,000). Incubate for 1 hr at RT.

5. **Wash:** 6 x 5 min at RT with TBST.
6. **Image** on the ChemiDoc™ MP Imaging System with Image Lab™ Touch Software. Configure a multichannel imaging protocol using the **Rhodamine** option under **Application** > **Blots** for the hFAB Rhodamine Antibody and the **StarBright B700** option under **Application** > **Blots** for the Starbright Blue 700 Secondary Antibody (for Image Lab Touch Software, version 2.0 and later software releases).

Ordering Information

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| 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 |

Related Products

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| 12004157 | Goat Anti-Mouse IgG StarBright Blue 700 , 5 x 400 μ l |
| 12004158 | Goat Anti-Mouse IgG StarBright Blue 700 , 400 μ l |
| 12004159 | Goat Anti-Mouse IgG StarBright Blue 700 , 80 μ l |
| 12004160 | Goat Anti-Rabbit IgG StarBright Blue 700 , 5 x 400 μ l |
| 12004161 | Goat Anti-Rabbit IgG StarBright Blue 700 , 400 μ l |
| 12004162 | Goat Anti-Rabbit IgG StarBright Blue 700 , 80 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 |
| 1610373 | Precision Plus Protein™ All Blue Standards , 500 μ l |

Precision Plus Protein Standards are sold under license from Life Technologies Corporation, Carlsbad, CA for use only by the buyer of the product. The buyer is not authorized to sell or resell this product or its components.

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