

Western Blotting

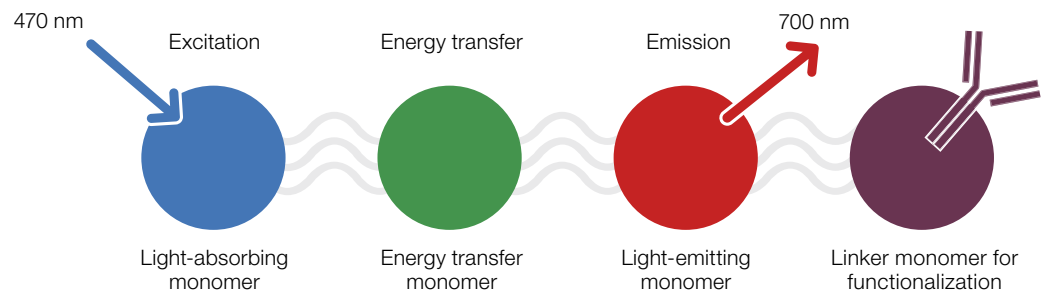


- Ideal for multiplex fluorescent western blotting
- Exceptional brightness
- Minimal background

Fluorescent Western Blotting Antibodies— Experience Unmatched Sensitivity

StarBright™ Blue 700 Secondary Antibodies Outperform Traditional Fluorescent Labels

StarBright is Bio-Rad's new ultrasensitive fluorescent label that is ideal for multiplex fluorescent western blotting. Each StarBright molecule is a condensed polymer made of multiple light-absorbing and -emitting monomer units, which makes it exceptionally bright (high quantum yield) compared to other traditional fluorophores.



Schematic representation of a StarBright label conjugated to an antibody. The light-absorbing monomer is excited at 470 nm and, via an efficient intramolecular energy transfer process, the energy is transmitted to a light-emitting monomer that emits at 700 nm. The linker monomer is used for antibody conjugation.

Key Advantages of StarBright Blue 700 Secondary Antibodies

- **Easy multiplexing** — can be used with other infrared dye-labeled antibodies or other common fluorophores to detect multiple proteins on the same blot. Bio-Rad's stain-free technology and/or hFAB™ Rhodamine Anti-Housekeeping Protein Primary Antibodies can be used for normalizing protein loading amounts
- **Shorter exposure time** — exceptional brightness leads to orders of magnitude shorter exposure times (seconds) compared to regular fluorescent dyes (minutes)
- **High signal with minimum noise** — emit in the near infrared range of the spectrum (excitation/emission: 470/700 nm), where there is minimal background, and can be used with Bio-Rad's new ChemiDoc™ MP Imaging System
- **Low nonspecific binding** — conjugated to highly cross-adsorbed antibodies

Cross-Adsorption of StarBright Antibodies

StarBright Antibody	Cross-Adsorbed against Serum Antibody Protein
Goat anti-mouse IgG StarBright Blue 700	Bovine, goat, human, rabbit, and rat
Goat anti-rabbit IgG StarBright Blue 700	Bovine, goat, human, mouse, and rat

Multiplexing to Detect Three Targets

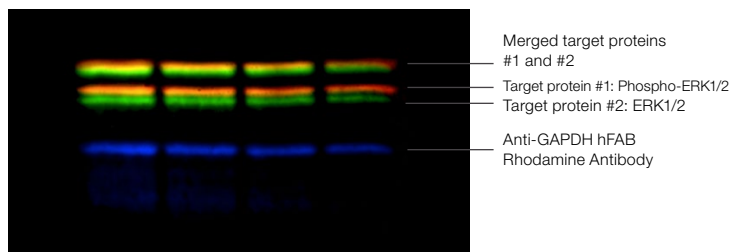


Composite blot image for triplex western blotting.

Target Protein	Primary Antibody	Secondary Antibody	Exposure Time, sec
#1: ATG7	Rabbit anti-human ATG7 (78 kD)	Goat anti-rabbit IgG StarBright Blue 700	6.9
#2: AKR1C2	Mouse anti-human AKR1C2 (36 kD)	Goat anti-mouse IgG DyLight 800	300
Housekeeping*	Anti-tubulin hFAB Rhodamine Antibody (50 kD)	No need for a secondary antibody	141.9

* In addition to or in place of housekeeping protein detection, a stain-free gel image from the same experiment can be used for total protein normalization.

Posttranslational Modifications



Composite blot image for triplex western blotting to detect posttranslational modifications such as phosphorylation of a target protein. Multiplexing to detect unmodified target protein, the phospho-target, and a housekeeping protein all in one blot.

Target Protein	Primary Antibody	Secondary Antibody
#1: Phospho-ERK1/2	—	Goat anti-mouse IgG StarBright Blue 700
#2: ERK1/2	—	Goat anti-mouse IgG DyLight 800
Housekeeping*	Anti-GAPDH hFAB Rhodamine Antibody (50 kD)	No need for a secondary antibody

* In addition to or in place of housekeeping protein detection, a stain-free gel image from the same experiment can be used for total protein normalization.

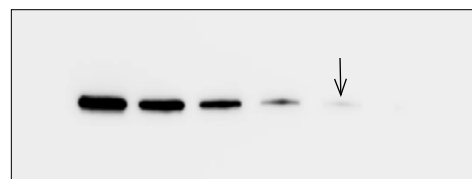
Better Data in Less Time



DyLight 800: 305 sec
No binning



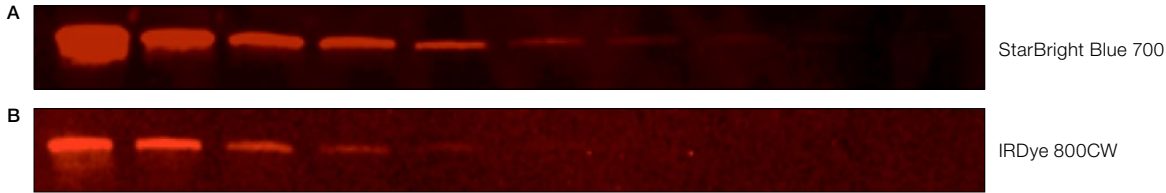
StarBright Blue 700: 10 sec
No binning



Clarity Max™ Western ECL Substrate: 8 sec
2 x 2 binning

StarBright Blue 700 Secondary Antibodies offer sensitivity at least comparable to high-end ECL substrates or better. Exposure times for StarBright Antibodies are much shorter than those for traditional fluorescent antibodies.

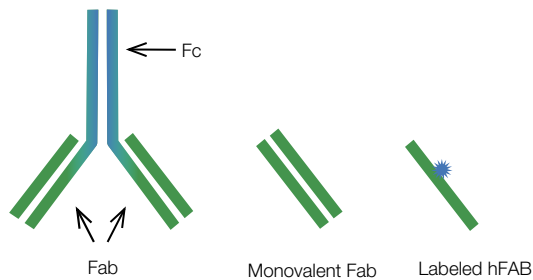
StarBright Blue 700 and IRDye 800CW Sensitivity



Comparison of StarBright Blue 700 and IRDye 800CW sensitivity using the ChemiDoc MP Imaging System. **A**, dilution series of maltose binding protein detected with StarBright Blue 700 and the ChemiDoc MP Imaging System; **B**, identical dilution series detected with IRDye 800CW and a competitor imaging system.

hFAB Rhodamine Antibodies Are Ideal for Housekeeping Protein Normalization

Never worry about species cross-reactivity again with Bio-Rad's unique one-step housekeeping protein primary antibodies. There is no need for a secondary antibody.



Schematic representation of the creation of a labeled hFAB Rhodamine Antibody starting from an IgG molecule.

Key Advantages of hFAB Rhodamine Antibodies

- **Easy** — human Fab fragment directly labeled with a rhodamine derivative; enables easy detection of housekeeping proteins (human, mouse, and rat actin/tubulin/GAPDH) without the need for a secondary antibody
- **Innovative** — created using the HuCAL[®] Human Combinatorial Antibody Library technology and can be used with other primary or secondary antibodies from different species without cross-reactivity
- **Flexible** — can be used for multiplexing with other antibodies, including StarBright Blue 700 and DyLight 800, to detect target proteins and housekeeping protein on the same western blot

Product Formats

Go to bio-rad.com/web/StarBrightAb and bio-rad.com/web/HKPAb for more information about product formats.



Goat Anti-Mouse IgG StarBright Blue 700
80 µl, 400 µl, 5 x 400 µl



Goat Anti-Rabbit IgG StarBright Blue 700
80 µl, 400 µl, 5 x 400 µl



hFAB Rhodamine Actin, Tubulin, and
GAPDH Anti-Housekeeping Antibodies
40 µl, 200 µl

Fluorescent Western Blotting

Go to bio-rad.com/web/WesternBlotting for more information about fluorescent western blotting.



Ordering Information

Catalog # Description

StarBright Blue 700 Secondary Antibodies

12004157 Goat Anti-Mouse IgG StarBright Blue 700, 5 x 400 µl
12004158 Goat Anti-Mouse IgG StarBright Blue 700, 400 µl
12004159 Goat Anti-Mouse IgG StarBright Blue 700, 80 µl
12004160 Goat Anti-Rabbit IgG StarBright Blue 700, 5 x 400 µl
12004161 Goat Anti-Rabbit IgG StarBright Blue 700, 400 µl
12004162 Goat Anti-Rabbit IgG StarBright Blue 700, 80 µl

hFAB Rhodamine Antibodies

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

Related Products

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
Multiple PrecisionAb™ Validated Western Blotting Antibodies

Visit bio-rad.com/web/StarBrightAb, bio-rad.com/web/HKPAbs,
and bio-rad.com/web/PrecisionAb for more information.

DyLight is a trademark of Thermo Fisher Scientific Inc. ECL is a trademark of GE Healthcare. HuCAL is a trademark of MorphoSys AG. IRDye is a trademark of LI-COR, Inc. Tween is a trademark of ICI Americas Inc.

Clarity Max Western ECL Substrate is manufactured by Cyanagen Srl and is the subject of patent application numbers US7855287, EP1950207, US9040252, AU2011202658, CA2742025, US8129136, and EP1962095, together with other equivalent granted patents and patent applications in other countries like CN102313732.

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