

TidyBlot Western Blot Detection Reagent:HRP

Solve 2 western blotting problems with 1 vial of TidyBlot

TidyBlot Reagent:HRP is a western blotting detection reagent that specifically binds to native (non-reduced) antibodies. In contrast to conventional secondary antibodies, it enables the detection of immunoblotted target protein bands without interference from denatured IgG.

Advantages of TidyBlot Reagent over Conventional Secondary Antibodies:

- **Detect only what matters** – TidyBlot Reagent exclusively binds to native non-denatured antibodies and not to any IgGs present in your immunoprecipitate or tissue lysate
- **Convenient** – simply substitute your conventional secondary antibody for TidyBlot Reagent
- **Broad species coverage** – detect a variety of monoclonal and polyclonal antibodies; no need to buy multiple HRP conjugated secondary antibodies

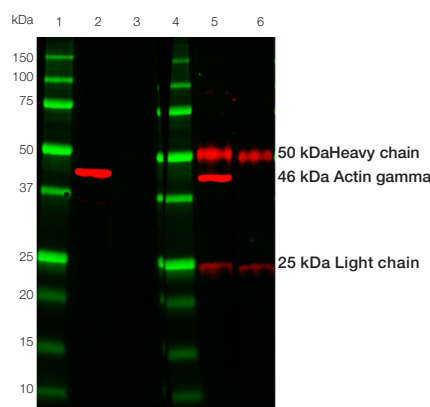


Fig. 1. Comparison of HRP conjugated TidyBlot Reagent (lanes 2, 3) to a standard mouse IgG (heavy and light chain) secondary antibody (lanes 5, 6). In contrast to the secondary antibody, which detected the IgG heavy chain at ~50 kDa and the IgG light chain at ~25 kDa, TidyBlot Reagent only bound to the Mouse Anti-Actin Gamma Antibody (cat. #VMA00049; 46 kDa) used for detection (lane 2). Lanes 3 and 6 are secondary only controls.

Note: TidyBlot Reagent is HRP conjugated and the western blot bands (red) and protein standards (green) have been pseudocolored.

BIO-RAD

Detect Your Protein of Interest without Interference from IgG Chains

Detecting your protein of interest by western blotting, in tissue lysates (**problem 1**) or immunoprecipitation (IP) samples (**problem 2**), can be challenging.

The IgGs/antibodies, eluted off the IP beads along with your protein of interest, become denatured during the IP sample preparation procedure. They are detected by conventional heavy and light chain secondary antibodies used in western blotting experiments. This binding results in the visualization of two distinct bands on your western blot; the IgG heavy chain at ~50 kDa and the IgG light chain at ~25 kDa (Figure 1).

Solve Two Problems with TidyBlot Reagent

Problem 1 - Western blot detection of IP samples:

- Protein of interest/immunoprecipitated protein has a molecular weight of ~50 kDa – heavy-chain will mask detection of the protein of interest
- Protein of interest/immunoprecipitated protein has a molecular weight of ~25 kDa – light-chain will mask detection of the protein of interest

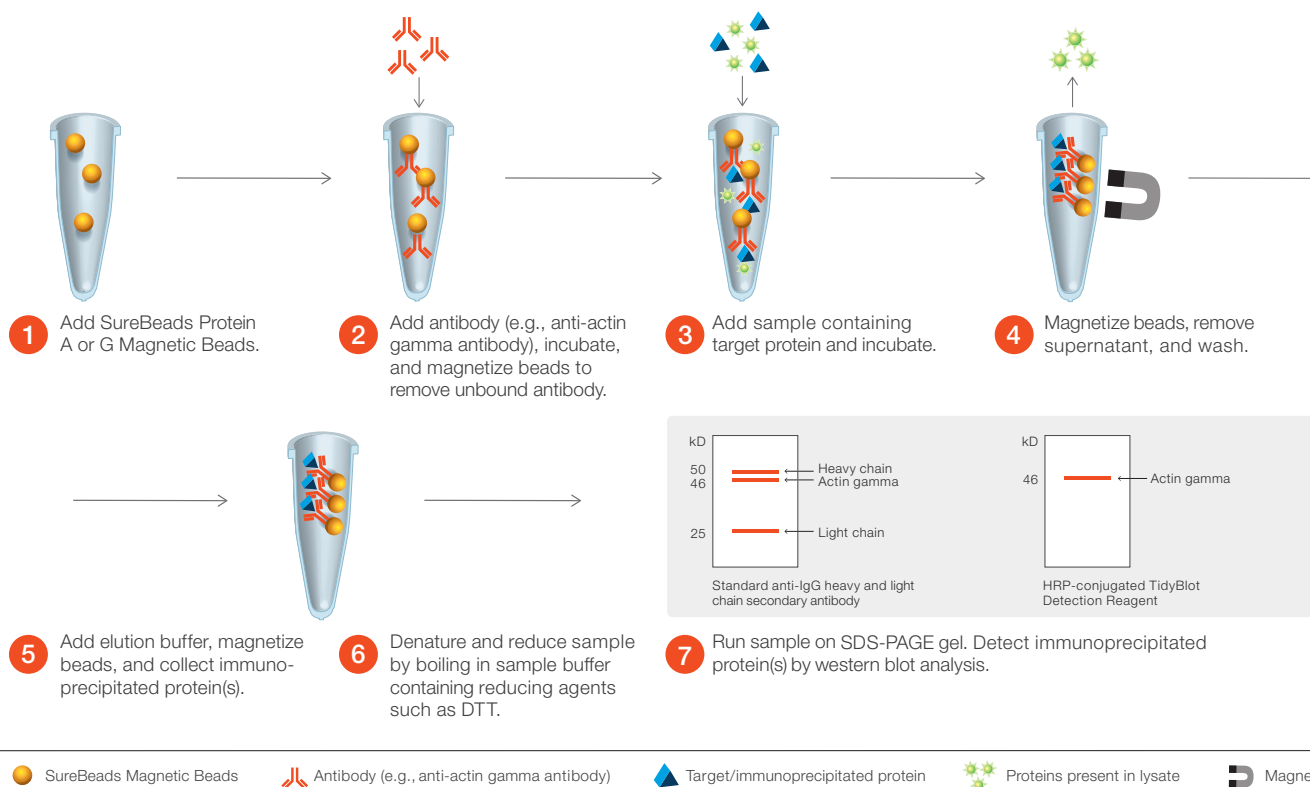


Fig. 2. Overview of actin gamma IP procedure using SureBeads Magnetic Beads followed by western blot detection of the IP sample with an anti-actin gamma primary antibody and a standard secondary antibody or TidyBlot Reagent. Using TidyBlot Reagent enables the detection of actin gamma without interference from the IgG heavy chain; the molecular weights of actin-gamma (46 kDa) and the IgG heavy chain (50 kDa) are very close, which often results in masking of the actin gamma band by the IgG heavy chain.

Solution - TidyBlot Reagent

TidyBlot HRP conjugated Western Blot Detection Reagent enables the detection of your protein of interest, without interference from IgG heavy and light chains, by only binding to the native primary antibody during the incubation of the western blot.

Western Blot Detection of Tissue Samples

Endogenous immunoglobulins in tissue lysate or immunoprecipitation samples, can be detected by conventional secondary antibody detection reagents. This can be particularly challenging in certain tissue types, such as thymus (Figure 3), which contain a significant number of immunoglobulins that become denatured and reduced during the sample preparation and SDS-PAGE stages of western blotting protocols.

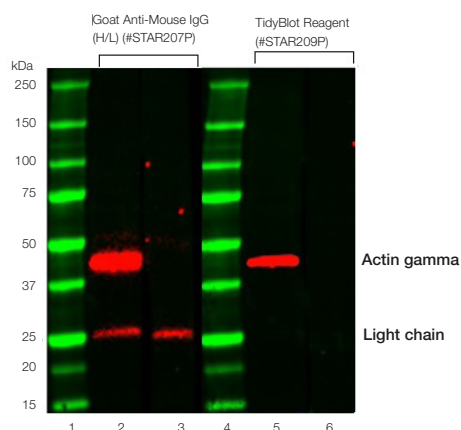


Fig. 3. Comparison of actin gamma detection in mouse thymus lysate using Goat Anti-Mouse IgG (H/L) or TidyBlot Reagent as secondary detection reagents.

Mouse Anti-Actin Gamma PrecisionAb Antibody (#VMA00049) was used in lanes 2 and 5. Lanes 3 and 6 are secondary only controls. HRP conjugated Goat Anti-Mouse IgG (H/L) Secondary Antibody (#STAR207P) was used in lanes 2 and 3. TidyBlot Reagent (#STAR209P) was used as the secondary reagent in lanes 5 and 6.

Note: TidyBlot Reagent is HRP conjugated and the western blot bands (red) and protein standards (green) have been pseudocolored.

Problem 2 - Western blot detection of tissue samples rich in endogenous immunoglobulins:

- Additional bands from binding of the secondary antibody to endogenous immunoglobulins (for example, visualization of the IgG light chain (Figures 3 and 4))
- Nonspecific secondary antibody binding resulting in high background staining

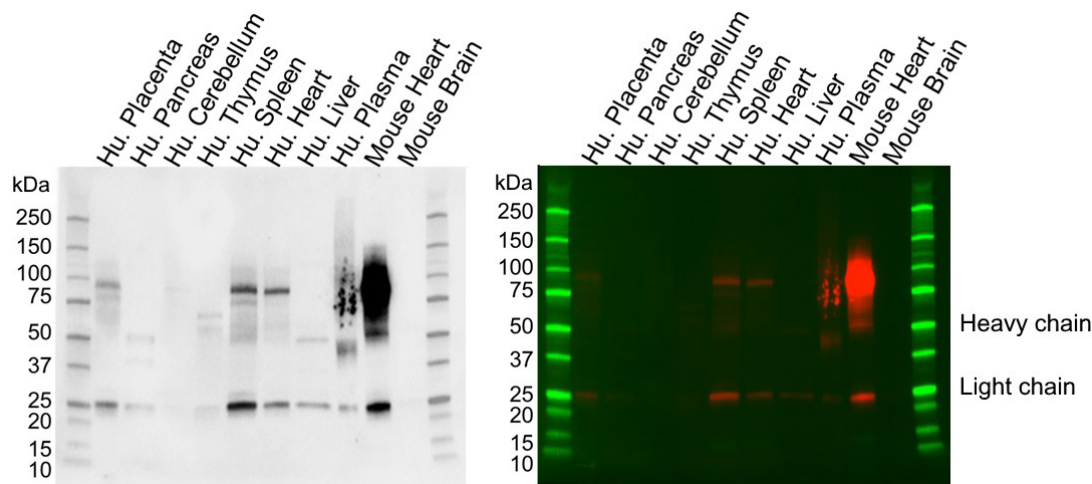


Fig. 4. Background staining in different tissue lysates using Immun-Star Goat Anti-Mouse (GAM)-HRP Antibody.

Note: TidyBlot Reagent is HRP conjugated and the western blot bands (red) and protein standards (green) in the right hand side image have been pseudocolored.

Solution - TidyBlot Reagent

As TidyBlot Reagent only binds to native non-denatured antibodies, trouble-free western blot detection of tissue lysates can be performed (low background risk).

Use TidyBlot Reagent for the Generation of Clean, Publication Quality, Western Blot Data.

Detect a Variety of Primary Antibodies

TidyBlot Reagent is very versatile as it is compatible with a variety of both monoclonal and polyclonal primary antibodies (Figure 5). In addition to convenience, you do not need to purchase multiple dedicated HRP conjugated secondary antibodies when using this reagent.

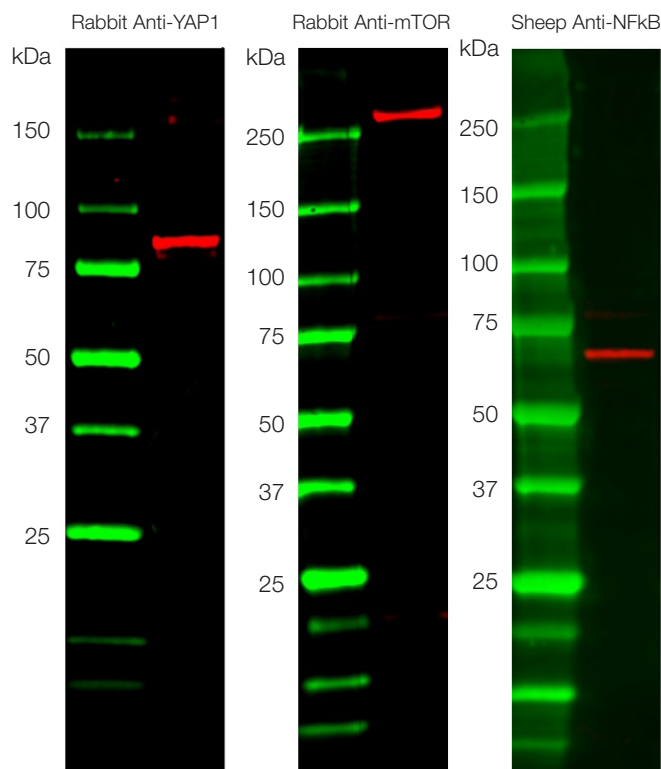


Fig. 5. Detection of goat, rabbit and sheep IgG polyclonal antibodies with TidyBlot Reagent. HeLa (YAP1 and mTOR) and HEK293 (NFkappaB p65) cell lysates were run on SDS-PAGE and transferred onto PVDF membranes. Goat Anti-YAP1 (#VPA00104), Rabbit Anti-mTOR (#VPA00174) and Sheep Anti-NFkappaB p65 (#VPA00015) antibodies were added at a dilution of 1/1000. Detection was performed with TidyBlot Reagent at a 1/200 dilution. All primary antibodies are part of the western blot validated PrecisionAb Antibody range.

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Compatibility with Antibodies

TidyBlot Reagent detects the IgG monoclonal and polyclonal antibodies listed below:

| Species | Monoclonal Isotypes | Polyclonal |
|---------|--|------------|
| Bovine | IgG2 | Compatible |
| Goat | IgG2 | Compatible |
| Human | IgG1, IgG2, IgG4 | Compatible |
| Mouse | IgG2a, IgG2b, IgG3, IgG1: affinity for IgG1 varies and may not be strong: affinity should therefore be tested on an antibody by antibody basis by performing dot blot analysis | Compatible |
| Rabbit | Total IgG | Compatible |
| Rat | IgG2c | Compatible |
| Sheep | IgG2 | Compatible |

When using mouse IgG1 monoclonal antibodies, it is recommended to perform a dot blot to determine compatibility, since TidyBlot Reagent might not detect all mouse IgG1 antibodies. Bio-Rad's HRP conjugated Rat Anti-Mouse Kappa Light Chain Specific Antibody, clone OX-20 (#MCA152P), may provide a solution if you are looking to detect immunoprecipitated proteins of ~50 kDa without masking by heavy chains. We also offer a comparable product for rabbit; Mouse Anti-Rabbit Light Chain Secondary Antibody, clone SB62a (#MCA6003P), an HRP conjugated secondary antibody that does not bind to rabbit IgG heavy chains.

Ordering Information

| Catalog # | Pack Size (ml) | Approx. No. Blots* |
|-----------|----------------|--------------------|
| STAR209P | 0.5 | 20 |
| STAR209PA | 1 | 40 |
| STAR209PT | 0.05 | 2 |

*Assuming a 1/200 dilution: 25 µl in 5 ml milk protein.

Visit bio-rad-antibodies.com/tidyblot for more information.

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