

# Frequently Asked Questions

IP/WB

## TidyBlot Western Blot Detection Reagent:HRP

### 1. What is TidyBlot Western Blot Detection Reagent:HRP?

TidyBlot Reagent (catalog #STAR209P, #STAR209PA and #STAR209PT) is a proprietary HRP conjugated secondary detection reagent, which only detects native IgG antibodies. It is not an antibody but can be used like a secondary antibody in western blot experiments.

### 2. What are TidyBlot's benefits over a conventional secondary antibody?

**TidyBlot has three advantages compared to conventional HRP conjugated (heavy and light chain) secondary antibodies**

- Detects only what matters — TidyBlot exclusively binds to native non-denatured antibodies and not to any IgGs present in your immunoprecipitate (IP) or lysate. You can therefore detect your protein of interest without obstruction by IgG heavy and light chains, and produce background free western blots
- Convenience — just substitute your conventional secondary antibody for TidyBlot
- Broad species compatibility — TidyBlot detects a variety of monoclonal and polyclonal antibodies. No need to buy several HRP conjugated secondary antibodies

### 3. What is the recommended dilution range?

TidyBlot is a substitute for a HRP conjugated secondary antibody and is therefore used in the same manner. The recommended TidyBlot dilution range is 1/40 to 1/400 (depending on the abundance of the protein of interest and the substrate used). In our experience a 1/200 dilution of TidyBlot in 5% non-fat milk yields more than 20 (#STAR209P) or 40 (#STAR209PA) mini western blots when using Clarity Western ECL Blotting Substrate.

### 4. How do I use TidyBlot and what should I be aware of?

TidyBlot preferentially binds to the native antibody used during the western blot procedure rather than the SDS-denatured/reduced form present in lysates/immunoprecipitates. Best results are therefore achieved by fully denaturing the immunoprecipitate/lysate (consider the duration of sample boiling to reduce IgG disulphide bonds and the type/concentration of reducing agent). TidyBlot is used in the same manner as conventional HRP conjugated secondary antibodies.

### 5. What antibody types is TidyBlot compatible with?

TidyBlot is compatible with the below listed IgG monoclonal and polyclonal antibodies:

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

TidyBlot is not known to detect IgA, IgD, IgE and IgM antibodies.

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## 6. What if I want to use the TidyBlot Reagent with a mouse IgG1 antibody?

If using a mouse IgG1 antibody, perform a dot blot to determine compatibility. The TidyBlot Reagent might not detect mouse IgG1. In that case you could try our HRP conjugated Rat Anti-Mouse Kappa Light Chain Specific Antibody, clone OX-20 (#MCA152P). This antibody does not detect mouse IgG heavy chains and thereby allows you to detect immunoprecipitated proteins of ~50 kDa without obstruction by heavy chains.

## 7. How many blots can I perform with one vial of the TidyBlot Reagent?

**One vial of TidyBlot enables you to perform 2 (50 µl, #STAR209PT), 20 (0.5 ml, #STAR209P) or 40 (1 ml, #STAR209PA) mini western blots;** assuming a 1/200 dilution in 5% non-fat milk using Clarity Western ECL Blotting Substrate. As is necessary, for every secondary detection reagent, you will need to establish the optimal dilution by performing a dilution series.

## 8. Can I purchase TidyBlot conjugated to other enzymes or fluorophores?

TidyBlot is currently only available conjugated to HRP. Unfortunately, we do not offer TidyBlot in other formats.

## 9. Can I purchase the TidyBlot in bigger size or in bulk?

Yes, it can be purchased in bulk as a multiple of 0.5 ml (#STAR209P) and 1 ml (#STAR209PA). Please contact your local sales representative for a bulk quote and delivery time.

## 10. Why are the TidyBlot western blot data in color when the product is HRP conjugated?

TidyBlot western blot data have been generated using the Bio-Rad ChemiDoc MP System rather than X-ray film. The system has various filters for the detection of fluorophores and other detection reagents based on their excitation and emission wavelengths. Each reagent is assigned a color (red, green or blue) when visualizing the blot with the ChemiDoc Imaging Software. In the case of the TidyBlot data, the chemi channel was used to detect HRP chemiluminescence and assigned the color red. Protein standards (Bio-Rad Precision Plus Protein All Blue Protein Standards) were detected in the DyLight 650 channel and assigned the color green\*.

\* Please note that these pseudocolors are not a reflection of emission colors

## 11. What other related secondary detection reagents does Bio-Rad offer?

We offer a HRP conjugated Rat Anti-Mouse Kappa Light Chain Specific Antibody, clone OX-20 (#MCA152P). This antibody does not detect mouse IgG heavy chains and thereby allows you to detect immunoprecipitated proteins of ~50 kDa without obstruction by heavy chains. We also offer a comparable product for rabbit; Mouse Anti-Rabbit Light Chain Antibody, clone SB62a (#MCA6003P), which is a HRP conjugated secondary antibody that does not bind to rabbit IgG heavy chains.

## 12. Where can I find information about Bio-Rad's IP and western blot tested antibodies?

Our entire catalog of over 700 IP tested primary antibodies can be found at:  
**[bio-rad-antibodies.com/immunoprecipitation-antibodies-kits-reagents](http://bio-rad-antibodies.com/immunoprecipitation-antibodies-kits-reagents)**

Bio-Rad offers over 800 western blotting tested antibodies, including PrecisionAb Antibodies:

**[bio-rad-antibodies.com/precisionab](http://bio-rad-antibodies.com/precisionab)**  
**[bio-rad-antibodies.com/western-blotting-antibodies-kits-reagents](http://bio-rad-antibodies.com/western-blotting-antibodies-kits-reagents)**

For more information, please go to **[bio-rad-antibodies.com/tidyblot](http://bio-rad-antibodies.com/tidyblot)**, contact your local sales representative or our technical support team **[bio-rad-antibodies.com/technical](http://bio-rad-antibodies.com/technical)** for more information.

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