abcam

Product datasheet

VeriBlot for IP Detection Reagent (HRP) ab131366

★★★★★ 7 Abreviews 81 References 6 Images

Overview

Product name	VeriBlot for IP Detection Reagent (HRP)
Conjugation	HRP
Tested applications	**Suitable for:** WB
General notes	VeriBlot for IP Detection Reagents are immunoblotting reagents that enable the trouble-free detection of immunoblotted target protein bands, without interference from denatured IgG. This allows to detect the (co-)immunoprecipitated protein without masking by the IgG heavy (50 kDa) and light chains (25 kDa). In general, this interference tends to originate from secondary antibodies which recognize primary antibodies released with the antigen during the immunoprecipitation procedure or endogenous IgGs from the lysate itself. VeriBlot for IP detection reagents only recognize native (non-reduced) antibodies and therefore the detection of heavy and light chains is highly minimized, if the immunoprecipitate is fully reduced. Number of blots: **At least 20** (based on a 1:200 dilution in 5 ml milk). **Important protocol notes (This information is available in Chinese here)** 1. The VeriBlot for IP Detection Reagent (HRP) detects the following IgG polyclonal and monoclonal antibodies:

Species	Monoclonal Isotype(s)
Bovine	IgG_2
Goat	IgG_2
Human	IgG_1, IgG_2, IgG_4
Mouse	IgG_{2a}, IgG_{2b}, IgG_3 ***Note:*** *If using mouse IgG_1, perform a dot blot to determine compatibility. VeriBlot for IP Detection Reagent (HRP) might not detect mouse IgG_1.*
Rat	IgG_{2C}
Rabbit	Total IgG
Sheep	IgG_2

2. The VeriBlot for IP Detection Reagent (HRP) preferentially detects the non-reduced form over the reduced, SDS-denatured forms.

3. IP sample should be completely reduced/denatured before loaded onto a western blot. Boil samples for 5-10 minutes in SDS sample buffer with a increase in SDS amount if required.

4. Milk should be used as the blocking protein for the immunoblot.

Note: If denatured and blotted IgG are not clearly detected, the following steps may be used to increase the amount of denatured IgG in the sample:

- Increase the concentration of reducing agent
- Boil sample to aid in reduction of IgG disulfide bonds
- Use dentaturing electrophoresis conditions

A full troubleshooting guide is available here.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C.
Storage buffer	Constituent: 1% MOPS

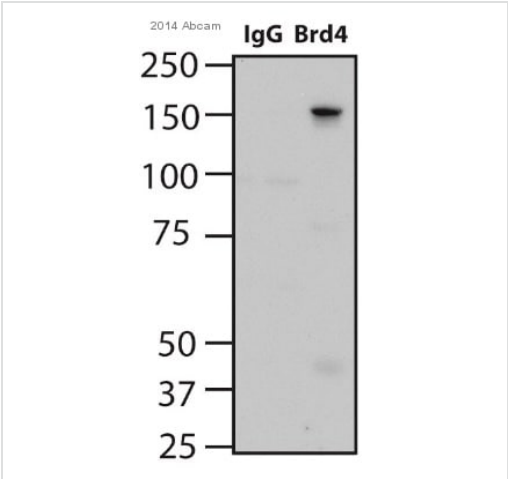
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab131366 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (3)	1/40 - 1/4000. The dilution will depend on the sensitivity of the HRP substrate. The dilution range recommended is 1:40 - 1:4000. Based on a 1:200 dilution (25 µL) in 5 ml milk researchers can perform 20 western blots. This product is recommended for the western blot detection of IP samples. Make sure the lysates are reduced and denatured completely

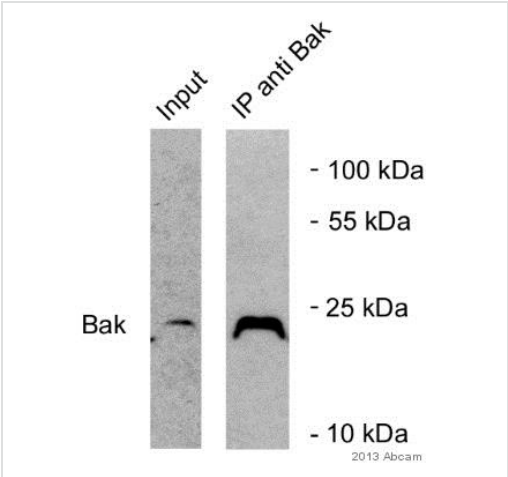
Images



Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

This image is courtesy of an anonymous Abreview.

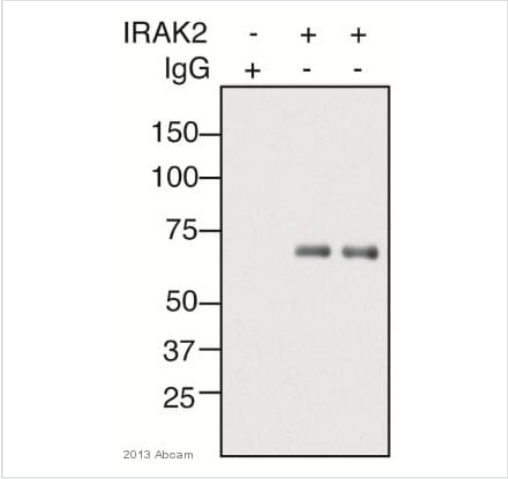
ab128874 Immunoprecipitating Brd4 in human HEK293 whole cell lysate. 1000µg of cell lysate was incubated with primary antibody (1µg/mg in 50 mM Tris) and matrix (Protein G) for 16 hours at 4°C. For western blotting a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/10000) was used to confirm successful immunoprecipation.



Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

This image is courtesy of an Abreview submitted by Christian Marx.

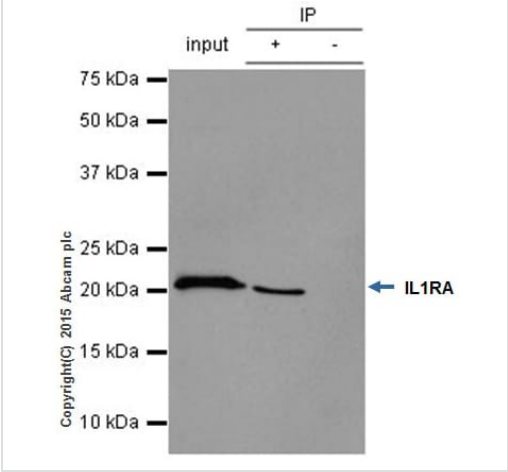
ab32371 immunoprecipitating Bak in human HCT116 p53-/- whole cell lysate. 100µg of cell lysate was incubated with primary antibody (1/100) and matrix (Protein A/G) for 4 hours at 4°C. For western blotting a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/2000) was used to confirm successful immunoprecipation.



Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

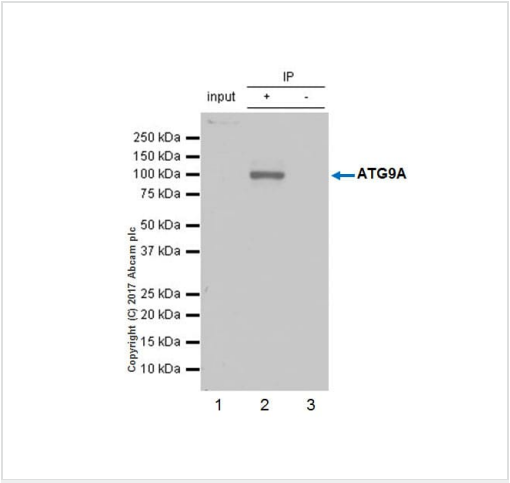
This image is courtesy of an anonymous Abreview.

ab6148 Immunoprecipitating IRAK2 in human HEK293 whole cell lysate. 1000µg of cell lysate was incubated with primary antibody (1 µg/mg) and matrix (Protein G) for 16 hours at 4°C. For western blotting a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/10000) was used to confirm successful immunoprecipation.



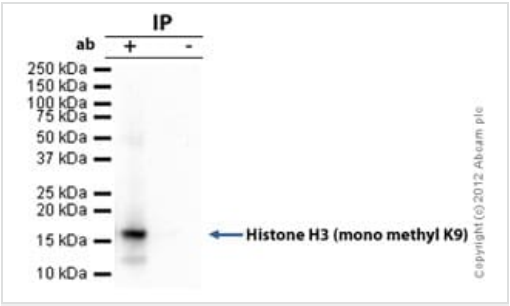
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

ab124962 (purified) at 1/20 immunoprecipitating IL-1RA in NIH/3T3 whole cell lysate.
Lane 1 (input): NIH/3T3 whole cell lysate (10µg)
Lane 2 (+): **ab124962** + NIH/3T3 whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab124962** in NIH/3T3 whole cell lysate.
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.
Blocking buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM /TBST.



Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

ab108338 (purified) at 1/20 dilution (2µg) immunoprecipitating ATG9A in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate.
Lane 1 (input): HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate 10µg
Lane 2 (+): **ab108338** & HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab108338** in HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate
For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution. No band in input lane is due to the boiled lysates
Blocking and diluting buffer: 5% NFDM/TBST.



Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366)

IP sample preparation: Histone H3 (mono methyl K9) was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Histone H3 (mono methyl K9) and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation. Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70^{o}C;

Western blot conditions: 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with **ab9045**.

Detection: VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution.

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