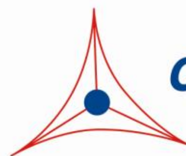
Product Manual

Pure-IP™ Western Blot Detection Kit

Catalog Number

PRB-5002	**20 blots**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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Creating Solutions for Life Science Research

Introduction

The technique of immunoprecipitation (IP) is used to isolate a protein of interest by capturing it with a resin-immobilized antibody specific for that protein. Typically, proteins are eluted from agarose beads under denaturing conditions that also release the IP antibody into solution, and then the protein of interest is detected by Western blotting. In many cases, the antibody used for IP must also be used for immunoblot detection of the protein of interest resulting in massive background due to the unwanted detection of heavy and light chain antibodies (Figure 1).

Cell Biolabs' Pure-IP™ Western Blot Detection Kit circumvents this common IP Western problem by using a proprietary HRP conjugate that cannot bind to contaminating heavy or light chains in the immunoblot lane of interest (Figure 1). The conjugate is only capable of detecting the antibodies that are properly folded and bound to the protein of interest during the immunoblot procedure resulting in a cleaner detection of the protein of interest.

Assay Principle

Cell Biolabs' Pure-IP™ Western Blot Detection Kit provides a format for reducing background when detecting an immunoprecipated protein of interest. The kit contains sufficient reagents for the performance of 20 immunoblots (8 cm x 8 cm). A cell or tissue lysate is prepared, and the IP is then performed. The protein of interest is eluted into SDS polyacrylamide Gel Electrophoresis (PAGE) sample buffer, separated by SDS PAGE, and transferred to nitrocellulose or PVDF membrane. The resulting immunoblot is then blocked with Pure-IP™ Blocking Solution and then incubated with the primary detection antibody diluted into Pure-IP™ Blocking Solution. After washing, the immunoblot is then incubated with the HRP conjugate diluted into Pure-IP™ Blocking Solution. After washing again, the immunoblot is developed by standard chemiluminescent or colorimetric techniques.

Related Products

1. AKR-190: RIPA Buffer
2. AKR-160: Rapid Antibody Purification Kit
3. AKR-110: Rapid GST Inclusion Body Solubilization and Renaturation Kit
4. AKR-172: Nuclear/Cytosolic Fractionation Kit
5. AKR-180: 5X Bacterial Protein Extraction Reagent (Tris)
6. AKR-181: 5X Bacterial Protein Extraction Reagent (Phosphate)
7. AKR-102: Phospho Antibody Stripping Solution
8. AKR-104: PhosphoBLOCKER™ Blocking Reagent
9. AKR-106: Phosphoprotein Purification Kit

Kit Components

1. 1000X HRP Conjugate (Part No. 50021C): One 200 µL vial
2. 20X Wash buffer (Part No. 50022B): One 120 mL bottle
3. Pure-IP™ Blocking Reagent (Part No. 50023B): One 7 gram bottle

Materials Not Supplied

1. IP reagents, such as protein A or G agarose beads
2. Antibodies for IP and/or Western blotting
3. SDS PAGE and Western transfer system
4. Nitrocellulose or PVDF membranes
5. Lysis buffer, such as 5X RIPA Buffer, with Protease Inhibitor Cocktail (Cat. #AKR-190)
6. Colorimetric or chemiluminescent Western Blot detection reagents

Storage

Store the HRP conjugate at -20°C. Store the remaining components at 4°C.

Preparation of Reagents

- 1X Wash Buffer: Dilute 20X Wash Buffer to 1X with deionized water and store at room temperature.
- Pure-IP™ Blocking Solution: Weigh out Pure-IP™ Blocking Reagent and add to 1X Wash Buffer at a final concentration of 1% (1 gram per 100 mL). Store at 4°C during use or at -20°C for long term storage.
- 1X HRP Conjugate Solution: Dilute the 1000X HRP Conjugate 1:1000 into Pure-IP™ Blocking Solution just before addition to an immunoblot.

Assay Protocol

Note: This protocol is written for use with 8 cm x 8 cm mini gels.

1. Prepare cell or tissue lysates in 1X RIPA Buffer or desired lysis buffer.
2. Perform immunoprecipitation procedure using your chosen antibody and Protein A or Protein G agarose beads.
3. Load samples onto a polyacrylamide gel and run electrophoresis until the dye front reaches the bottom of the gel.
4. Transfer to a nitrocellulose or PVDF membrane.
5. Remove the membrane from the transfer apparatus and place in 10 mL of Pure-IP™ Blocking Solution for one hour, with gentle shaking.
6. Dilute the primary antibody in 10 mL of Pure-IP™ Blocking Solution.

   Note: antibody dilutions should be determined by the user. Affinity purified antibody typically requires dilutions between 1-30 µg/mL.
7. Remove the Pure-IP™ Blocking Solution and add the diluted primary antibody.
8. Incubate for one to two hours at room temperature (or overnight at 4°C) with gentle shaking.

9. Wash the membrane three times for 10 minutes each time with 10 mL of 1X Wash Buffer.
10. Incubate the membrane in 10 mL of 1X HRP Conjugate Solution for 60 minutes at room temperature with gentle shaking.
11. Wash the membrane four times for 10 minutes each time with 10 mL of 1X Wash Buffer.
12. Develop blots with colorimetric or chemiluminescent Western blot detection reagents.
13. Expose membrane to film or CCD camera.

Example of Results

The following figure demonstrates typical results with the Pure-IP™ Western Blot Detection Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

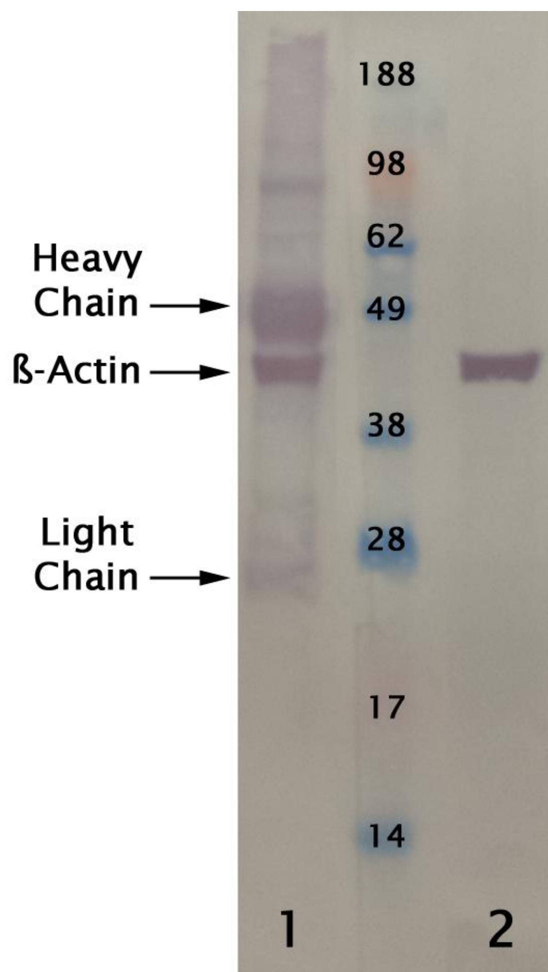

Figure 1. Detection of actin from HeLa whole cell lysate mixed with monoclonal antibody. Cell lysates from HeLa cells were prepared using RIPA buffer (AKR-190). 20 µg of HeLa whole cell lysate and 2 µg of monoclonal antibody were loaded per lane. Lane 1 was probed with goat anti-mouse HRP, while lane 2 was probed with 1X HRP Conjugate Solution from the Pure-IP™ Western Blot Detection Kit.

References

1. Rosenberg, Ian (2005). Protein analysis and purification: benchtop techniques. Springer. p. 520.
2. Phizicky E. M. and Fields S. (1995) *Microbiol Rev*. 59, 94-123.
3. Golemis E. (2002) Protein-protein interactions: A molecular cloning manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. ix, 682.

Warranty

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