SANTA CRUZ BIOTECHNOLOGY, INC.

ImmunoCruz™ IP/WB Optima B System: sc-45039

BACKGROUND

The ImmunoCruz™ IP/WB Optima product line provides a new and improved method for the detection of immunoprecipitated proteins via Western Blot (WB) analysis. When used as directed, IP/WB Optima effectively eliminates the detection of heavy and light chains of the IP antibody. Santa Cruz Biotechnology provides six unique detection systems that apply to IP/WB that use any combination of goat, rabbit and mouse antibodies as the IP and Western Blotting (WB) antibodies. Each kit contains the required IP matrix to precipitate the desired Ag-Ab complex and an HRP conjugated reagent that detects only the desired WB antibody. IP/WB Optima technology is of particular value for the analysis of cellular proteins that are expressed at very low levels and thus difficult to detect using conventional Western Blotting procedures.

PRODUCTS

ImmunoCruz™ IP/WB Optima B is an antigen detection system comprised of one each of 2.0 ml (25% v/v) Immunoprecipitation Matrix for goat and rabbit primary antibodies and 0.5 ml Western Blotting Detection Reagent for detection of mouse primary antibodies. WB dilution range is 1:1000–1:10000.

IMMUNOPRECIPITATION PROTOCOL

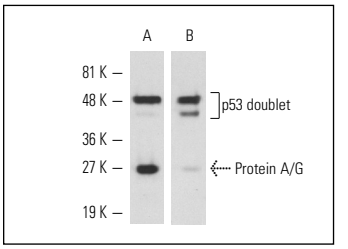
- Prepare a total cell lysate as described under the Western (Immuno-) Blotting procedure in the Protocols and Support Products chapter of the Santa Cruz Biotechnology catalog or visit our website at www.scbt.com
- Preclear whole cell lysate (optional): Use appropriate Preclearing Matrix (sold separately; Preclearing Matrix B-goat: sc-45053 or Preclearing Matrix B-rabbit: sc-45059). To approximately 1 ml of whole cell lysate or tissue extract in a 1.5 ml microcentrifuge tube, add 40-50 µl of the suspended (25% v/v) preclearing matrix. Incubate for 30 minutes at 4° C while rotating. Note: If the lysate was prepared from cells expressing Igs (i.e., spleen cells or cultured B cells), a preclearing step with Protein A/G agarose should also be performed 2-3 times to ensure complete removal of endogenous Igs.
- Pellet matrix via microcentrifugation at maximum speed for 30 seconds at 4° C. Without disturbing pellet, transfer desired supernatant (cell lysate) to new microcentrifuge tube. Store precleared lysate on ice and discard pellet.
- Formation of the IP antibody-IP matrix complex: To a microcentrifuge tube, add 40-50 µl of suspended (25% v/v) IP matrix, 1-5 µg of IP antibody and 500 µl of PBS. Optimal antibody amount should be determined by titration. Incubate at 4° C on a rotator for at least one hour. This step can be performed in parallel with the above preclearing step or performed the day before and allowed to incubate overnight at 4° C.
- After incubation of the IP antibody with the species specific IP matrix, pellet matrix via microcentrifugation at maximum speed for 30 seconds at 4° C. Carefully aspirate and discard supernatant.
- Wash pelleted matrix two times with 500 µl of PBS, each time repeating the above centrifugation and aspiration steps.
- Immunoprecipitation: After the final wash of the IP antibody-IP matrix complex, transfer lysate (100-1000 µg of total cellular protein) to the pelleted matrix and incubate at 4° C on a rotator for one hour to overnight.
- After incubation, microcentrifuge at maximum speed for 30 seconds at 4° C to pellet IP matrix. Aspirate and discard supernatant.
- Wash pelleted matrix 2-4 times with either RIPA buffer (more stringent) or PBS (less stringent), each time repeating the above centrifugation and aspiration steps.
- After final wash, aspirate and discard the supernatant and resuspend pellet in 40-50 µl of 2X reducing electrophoresis buffer. Boil samples for 2-3 minutes. Note: The immunoprecipitated sample must be completely reduced and denatured for IP/WB Optima to work properly.
- Perform a quick spin to pellet IP matrix, carefully load desired supernatant onto gel and immunoblot via standard methods. Detect WB antibody probe using the appropriate HRP conjugated ImmunoCruz™ reagent via standard incubation and detection protocols.

IMMUNOCRUZ™ IP/WESTERN BLOT REAGENTS

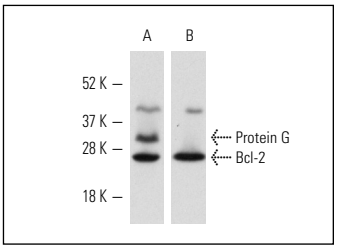
PRODUCT	CAT. #	USE	SPECIES OF IP ANTIBODY	SPECIES OF WB ANTIBODY
IP/WB Optima A	sc-45038	heterologous IP/WB	rabbit or mouse	goat
IP/WB Optima B	sc-45039	heterologous IP/WB	goat or rabbit	mouse
IP/WB Optima C	sc-45040	heterologous IP/WB	goat or mouse	rabbit
IP/WB Optima D	sc-45041	homologous IP/WB	goat	goat
IP/WB Optima E	sc-45042	homologous IP/WB	mouse	mouse
IP/WB Optima F	sc-45043	homologous IP/WB	rabbit	rabbit

ImmunoCruz™ IP/WB Optima reagents are optimized for primary antibody detection in Western Blot analysis of immunoprecipitates. IP/WB Optima technology is designed to detect the desired Western Blot probe antibody without detection of heavy and light chains of the IP antibody. Each kit contains 2.0 ml (25% v/v) Immunoprecipitation Matrix and 0.5 ml Western Blotting Detection Reagent. Western blotting reagents for heterologous IP/WB should be used at a dilution of 1:1000–1:10000. Western blotting reagents for homologous IP/WB should be used at a dilution of 1:1000–1:4000.

DATA



Immunoprecipitation of p53 from A-431 whole cell lysate using p53 (C-19): sc-1311 (goat polyclonal antibody) followed by Western blot analysis using p53 (DO-1): sc-126 (mouse monoclonal antibody). Note presence of Protein A/G band using Protein A/G PLUS-Agarose: sc-2003 conventional IP matrix (**A**) as compared to their absence using IP/WB Optima B: sc-45039 (**B**).



Immunoprecipitation of Bcl-2 from U-937 whole cell lysate using Bcl-2 (N-19)-G: sc-492-G (goat polyclonal antibody) followed by Western blot analysis using Bcl-2 (C-2): sc-7382 (mouse monoclonal antibody). Note presence of Protein G band using conventional IP reagent Protein G PLUS-Agarose: sc-2002 (**A**) versus the absence of Protein G using IP/WB Optima B: sc-45039 (**B**).

SELECT PRODUCT CITATIONS

1. Diamond, E., et al. 2006. Functional interactions between Dlx2 and lymphoid enhancer factor regulate Msx2. Nucleic Acids Res. 34: 5951-5965.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.