

## BACKGROUND

The ExactaCruz™ product line provides a new and improved method for the detection of immunoprecipitated proteins via Western Blot (WB) analysis. When used as directed, ExactaCruz™ 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. ExactaCruz™ 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

ExactaCruz™ C is an antigen detection system comprised of one each of 2.0 ml (25% v/v) Immunoprecipitation Matrix for goat and mouse primary antibodies and 0.5 ml Western Blotting Detection Reagent for detection of rabbit 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](http://www.scbt.com)
- Preclear whole cell lysate (optional): Use appropriate Preclearing Matrix (sold separately; Preclearing Matrix C-goat: sc-45054 or Preclearing Matrix C-mouse: sc-45060). 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 a new microcentrifuge tube. Store precleared lysate on ice and discard the 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.

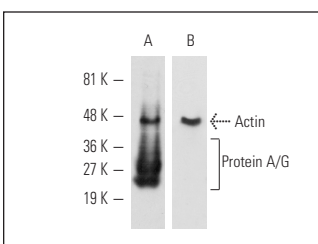
- After incubation, microcentrifuge at maximum speed for 30 seconds at 4° C to pellet IP matrix. Aspirate and discard supernatant.
- Wash pelleted matrix 2-4 times with either RIPA buffer (more stringent) or PBS (less stringent), each time repeating the above centrifugation and aspiration steps.
- After 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 ExactaCruz™ 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 ExactaCruz™ reagent via standard incubation and detection protocols.

## EXACTACRUZ™ IP/WESTERN BLOT REAGENTS

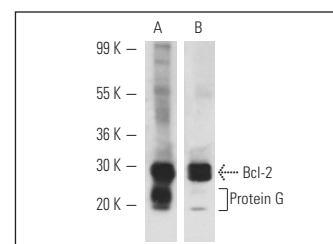
PRODUCT	CAT. #	USE	SPECIES OF IP ANTIBODY	SPECIES OF WB ANTIBODY
ExactaCruz™ A	sc-45038	heterologous IP/WB	rabbit or mouse	goat
ExactaCruz™ B	sc-45039	heterologous IP/WB	goat or rabbit	mouse
ExactaCruz™ C	sc-45040	heterologous IP/WB	goat or mouse	rabbit
ExactaCruz™ D	sc-45041	homologous IP/WB	goat	goat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

ExactaCruz™ reagents are optimized for primary antibody detection in Western Blot analysis of immunoprecipitates. ExactaCruz™ 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 Actin from Sol8 whole cell lysate using Actin (I-19): sc-1616 (goat polyclonal antibody) followed by Western blot analysis using Actin (C-11)-R: sc-1615-R (rabbit polyclonal 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 ExactaCruz™ C: sc-45040 (B).



Immunoprecipitation of Bcl-2 from U-937 whole cell lysate using Bcl-2 (100): sc-509 (mouse monoclonal antibody) followed by Western blot analysis using Bcl-2 (N-19): sc-492 (rabbit polyclonal antibody). Note presence of Protein G band using Protein A/G PLUS-Agarose: sc-2003 conventional IP matrix (A) as compared to their absence using ExactaCruz™ C: sc-45040 (B).

## 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.