SANTA CRUZ BIOTECHNOLOGY, INC.

ImmunoCruz™ IP/WB Optima E System: sc-45042

BACKGROUND

The ImmunoCruz™ product line provides a new and improved method for the detection of immunoprecipitated proteins via Western Blot (WB) analysis. When used as directed, ImmunoCruz™ 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. ImmunoCruz™ technology is of particular value for the analysis of cellular proteins that are expressed only at very low levels and thus difficult to detect using conventional Western Blotting procedures.

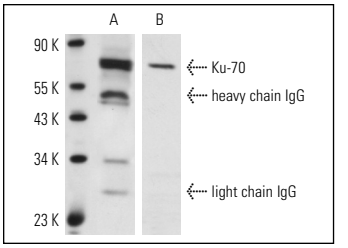
PRODUCTS

ImmunoCruz™ E is an antigen detection system comprised of a 2.0 ml (25% v/v) Immunoprecipitation Matrix for mouse primary antibodies, 0.5 ml Western Blotting Detection Reagent for detection of mouse primary antibodies and 250 ml (2x solution) ImmunoCruz™ E Dilution Reagent. WB Detection Reagent dilution range is 1:1000–1:10000.

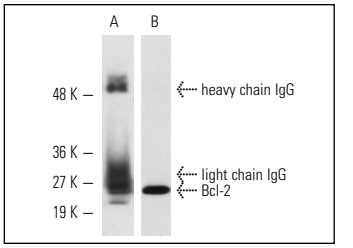
IMMUNOPRECIPITATION PROTOCOL

- 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
- Optional: Preclear with Preclearing Matrix E: sc-45056 (sold separately). 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 ImmunoCruz™ to work properly.
- Perform a quick spin to pellet IP matrix, carefully load desired supernatant onto gel and electrophorese according to standard protocols. Transfer proteins from the gel to a nitrocellulose or PVDF membrane.
- After transfer, block/wash membrane with TBST (10x TBST: sc-24953) for 1 hour, changing TBST once half way through the incubation.
- Dilute WB antibody with 1x ImmunoCruz E Dilution Buffer (provided as 2 x solution, dilute to 1 x using diH_2O), add to membrane and incubate for 1-2 hours at room temperature. Do NOT incubate overnight at 4° C.
- After incubation, wash 3x with 1x TBST, 5 minutes per wash.
- Dilute 1x ImmunoCruz E Western Blot Reagent (1:1000-1:10000) with ImmunoCruz E Dilution Buffer (provided), add to membrane and incubate 1-2 hours at room temperature. Do NOT incubate overnight at 4° C.
- Wash membrane 3x with TBST, 5 minutes per wash.
- Wash membrane once with 1x TBS (10x TBS: sc-24951) for 5 minutes.
- Incubate membrane in Western Blot Luminol Reagent: sc-2048 according to Luminol data sheet.

DATA



Immunoprecipitation of Ku-70 from HeLa whole cell lysate using Ku-70 (E-5): sc-17789 (mouse monoclonal antibody) followed by Western blot analysis using Ku-70 (A-9): sc-5309 (mouse monoclonal antibody). Note presence of IgG heavy and light chains using bovine anti-mouse IgG-HRP conventional secondary antibody: sc-2380 (**A**) as compared to their absence using ImmunoCruz™ E: sc-45042 (**B**). Also note presence of TdT/Ku-70 complex below heavy chain IgG.



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 (C-2): sc-7382 (mouse monoclonal antibody). Note presence of IgG heavy and light chains using goat anti-mouse IgG-HRP conventional secondary antibody: sc-2005 (**A**) as compared to their absence using ImmunoCruz™ E: sc-45042 (**B**).

STORAGE

Store IP matrix and WB Reagents at 4° C and store ImmunoCruz E Dilution Reagent at room temperature. If Dilution Reagent solidifies, heat in warm water bath. **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.