



The Power to Quantify

santa cruz biotechnology, inc.

ExactaCruz™ Protocol



- Prepare a total cell lysate as described under Western (Immuno-) blotting procedure.
- Preclear whole cell lysate (optional): Use appropriate [Preclearing Matrix](#) for your kit (sold separately). To approximately 1 ml of whole cell lysate or tissue extract in a 1.5 ml microcentrifuge tube, add 40-50 μ l 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 IP matrix via microcentrifugation at maximum speed for 30 seconds at 4° C. Without disturbing pellet, transfer desired supernatant (precleared 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 μ l of suspended (25% v/v) IP matrix, 1-5 μ g of IP antibody and 500 μ l 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.

NOTE: It is necessary that the species of the IP antibody matches the species of the IP matrix included with each ExactaCruz™ kit. For example, when performing an IP with a mouse antibody, it must be incubated with the Mouse IP Matrix provided ([sc-45040](#) or [sc-45042](#)).

- 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 2 times with 500 μ l 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 μ g of total cellular protein) to the pelleted matrix and incubate at 4° C on a rotator for one hour to overnight.
- After incubation of the matrix and lysate, microcentrifuge at maximum speed for 30 seconds at 4° C to pellet. Aspirate and discard supernatant or alternatively keep supernatant for another IP or testing via western blot.
- Wash pelleted matrix 2-4 times with either RIPA Lysis Buffer: [sc-24948](#) (more stringent) or 1x 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 μ l of reducing 2x Electrophoresis Sample Buffer: [sc-24945](#). 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 and carefully load supernatant onto gel. Continue with electrophoresis as described under the Western (Immuno) Blotting procedure.
- At this stage it is essential that the immunoblotting (primary) antibody matches the species specificity of the HRP conjugated ExactaCruz™ detection reagent which is unique for each kit. Detect the immunoblotting (primary) antibody using the corresponding HRP conjugated ExactaCruz™ reagent and Western Blot Luminol Reagent: [sc-2048](#).

NOTE: When using [sc-45042](#) ExactaCruz™ E, the alternate immunoblotting protocol that is specific for this kit must be followed as described below in order to generate desired results.

ExactaCruz™ E Alternate Protocol

- 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 ExactaCruz™ E Dilution Buffer (provided), add to membrane and incubate for 1-2 hours at room temperature.
- After incubation, wash 3x with 1x TBST, 5 minutes per wash.
- Dilute ExactaCruz E Western Blot Reagent (1:1000-1:10000) with ExactaCruz E Dilution Buffer (provided), add to membrane and incubate 1-2 hours at room temperature.
- 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.