- Prepare a total cell lysate as described under Western blot procedure in protocol 1.
- Preclear whole cell lysate (optional step) as follows. To approximately 1 ml of whole cell lysate or tissue extract (see Whole Cell Lysates or Tissue Extract Tables), add 0.25 µg of the appropriate control IgG (corresponding to the host species of the primary antibody; see Control IgGs and IgG Conjugates), together with 20 µl of appropriate suspended (25% v/v) agarose conjugate (Protein A-Agarose, Protein G-Agarose, Protein A/G-Agarose, or Protein L-Agarose). Incubate at 4° C for 30 minutes.
- Pellet beads by centrifugation at 3,000 rpm (approximately 1,000xg) for 30 seconds at 4° C. Transfer supernatant (cell lysate) to a new microcentrifuge tube at 4° C.
- To 1 ml of the above cell lysate, or approximately 100–1000 µg of total cellular protein, add 10 µg of primary antibody agarose conjugate (i.e., 5 µl volume of packed beads) and incubate at 4° C for 1 hour to overnight with mixing.
- Alternatively, if primary antibody agarose conjugate is not available, incubate 1 ml cell lysate with 1–10 µl (i.e., 0.2–2 µg) primary antibody (optimal antibody concentration should be determined by titration) for 1–2 hours at 4° C. Add 20 µl of appropriate agarose conjugate suspension (Protein A-Agarose, Protein G-Agarose, Protein A/G-Agarose or Protein L-Agarose). Cap tubes and incubate at 4° C on a rocker platform or rotating device for 1 hour to overnight.
- Collect pellet by centrifugation at 3,000 rpm (approximately 1,000xg) for 30 seconds at 4° C. Carefully aspirate and discard supernatant.
- Wash pellet 2–4 times with either RIPA buffer (sc-24948) (more stringent) or PBS (Buffers and General Solutions) (less stringent), each time repeating centrifugation step above.
- After final wash, aspirate and discard supernatant and resuspend pellet in 40 µl of 2x electrophoresis sample buffer (sc-24945).
- Boil samples for 2–3 minutes. Load up to 5–10 µl of sample per 1.0 mm well width for gels of 0.75 mm thickness.
- Continue with electrophoresis and immunoblotting as described under Western blotting procedure in protocol 1.

NOTE: Depending on the secondary antibody that is used, 55 kDa and 27 kDa heavy and light IgG chains, respectively, of the primary antibody may be detected. These bands will be less pronounced if a primary antibody agarose conjugate is used in the above procedure or if ExactaCruz™ Reagents are used.