



The Power to Quantify

santa cruz biotechnology, inc.

Kinase Assay Protocol



- Remove medium from 100 mm cell culture plate (80–90% confluent monolayer) and wash once with PBS ([Buffers and General Solutions](#)).
- Add 1–3 ml ice cold RIPA buffer ([sc-24948](#)) to cell monolayer and incubate at 4° C for 10 minutes. (**NOTE:** the use of RIPA buffer may not be optimal for some kinases. Composition of lysis buffer may need to be optimized to maintain active kinase.)
- Disrupt cells by repeated passage through a 21-gauge needle and transfer to microcentrifuge or 15 ml conical centrifuge tube.
- Wash cell culture plate with addition of 1.0 ml ice cold RIPA buffer, 0.5% Triton X-100 (Triton X-100: [sc-29112](#)) and combine with original extract.
- Pellet cellular debris at 10,000xg for 10 minutes at 4° C. Transfer supernatant to a new microcentrifuge or 15 ml conical centrifuge tube at 4° C.
- Transfer 1.0 ml cell extract (supernatant from above step) to a 1.5 ml microcentrifuge tube. Add 1–10 µl (i.e., 0.2–2 µg) primary antibody (optimal antibody concentration should be determined by titration) and incubate for 1 hour 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 immunoprecipitates by centrifugation at 2,500 rpm (approximately 1,000xg) for 5 minutes at 4° C. Carefully aspirate and discard supernatant.
- Wash pellet four times with 1.0 ml RIPA buffer (more stringent) or PBS ([Buffers and General Solutions](#)) (less stringent), each time repeating centrifugation step above.
- Suspend pellet in 20 µl of the appropriate protein kinase assay buffer (e.g., 50 mM HEPES (HEPES: [sc-29097](#)), 0.1 mM EDTA (EDTA: [sc-29092](#)), 0.01% BRIJ® 35 (BRIJ® 35: [sc-29087](#)), 0.1 mg/ml BSA, 0.1% β-mercaptoethanol 0.15 M NaCl. Buffer composition will depend upon the kinase under study.
- Add 10–1000 ng peptide substrate. Peptide substrate concentration should be determined empirically for the substrate/enzyme/cell line used.
- Prepare 1 ml ATP mix: 930 µl appropriate protein kinase assay buffer, 6 µl 50 mM ATP, pH 7.0, 20 µl 2.0 M MgCl², and 44 µl [γ -³²P]-ATP [10 mCi/ml]. Add 10 µl ATP mix per sample and incubate for 20 minutes at 30° C. Place on ice.
- Terminate the reaction by adding an equal volume of 2x electrophoresis sample buffer ([sc-24945](#)) and boil samples for 2–3 minutes. After boiling, samples may be centrifuged to pellet the agarose beads (optional); the supernatant is analyzed. Analyze samples by SDS-PAGE and autoradiography. Unused samples may be stored at -20° C. Labeled peptides can be separated from unincorporated label by acid precipitation followed by collection on a filter and radioactivity determined by scintillation counting. Researchers may also choose to analyze immobilized peptides prepared by standard methods or offered commercially.