

**Certificate of Analysis****Product:** Normal Mouse Brain Whole Cell Lysate (Ready-to-Use)**Code:** W10-000-T004**Lot #** 20115**Size:** 500 µg (500 µl)**Physical State:** Liquid (frozen)**Total Protein Concentration:** 1.0 mg/ml (by Lowry Assay)**Buffer:** 1X SDS-PAGE Sample Buffer consisting of 62.5 mM Tris HCl, 2% SDS, 10% Glycerol and 0.005% bromophenol blue, pH 6.8.**Cell Type:** Brain - Mouse (mixed breed and sex)**Induction:** None (Control)**Background):** Ready-to-use whole cell lysates produced by Rockland Immunochemicals are derived from cell lines or tissues using highly refined extraction protocols to ensure exceptionally high quality, protein integrity and lot-to-lot reproducibility. All extracts are tested by SDS-PAGE using 4-20% gradient gels and immunoblot analysis using antibodies to key cell signaling components to confirm the presence of both high molecular weight and low molecular weight proteins.**Application(s):** Ready-to-use lysates are especially prepared as positive controls for separation by SDS-PAGE and subsequent western blot analysis. Lysates are prepared in denaturing buffer WITHOUT dissociating agents (i.e. no 2-mercaptoethanol or dithiothreitol has been added).**Preparation Method:** Tissues were washed exhaustively with PBS to remove blood and other debris. A lysate was prepared by homogenizing the tissue and washing the cells in cold PBS. Washed cells were incubated at 4° C in modified RIPA buffer containing 150 mM sodium chloride, 50 mM Tris Cl, pH 7.4, 1 mM EDTA, 1.0% NP-40, 0.25% sodium deoxycholic acid to lyse the cells. Protein integrity is ensured using a cocktail of protease inhibitors with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases (0.1 mM AEBSF HCl, 0.08 µM Aprotinin, 5 µM Bestatin, 1.5 µM E-64, 2 µM Leupeptin Hemisulfate and 1 µM Pepstatin A). The following phosphatase inhibitors were also added: 1 mM NaF and 1 mM Na<sub>3</sub>VO<sub>4</sub>. Cell debris was removed by centrifugation and membrane filtration. Protein concentration was determined by Lowry assay using a commercially available kit. The protein concentration was adjusted to 2 mg/ml and then an equal volume of 2X SDS-PAGE sample buffer was added.**Recommended Dilution(s):** Heat lysate to 95° C for 5 minutes and rapidly cool. If dissociating conditions are desired add reducing agent prior to heating. The recommended loading volume per lane is 10-20 µl depending on the size format of your gel.**Storage Conditions:** Store vial at -20° C or COLDER. For extended storage, aliquot contents to minimize freeze/thaw cycles. Expiration date is three (3) months from date of opening if stored at -20° C or (1) year from date of opening if stored at -70°C.**Custom Service(s):** Please inquire for nuclear and/or whole cell extracts from other unstimulated or stimulated cell lines or normal tissues in both research and bulk quantities. Custom lysates from researcher-provided cell lines are also prepared using our highly refined extraction protocols. Please contact our Technical Service staff for additional details.**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

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