

NBP2-27950 Protocol

Product Handling Protocol (NBP2-27950)

Lysate Preparation

Tissue specimens are homogenized in modified RIPA buffer to obtain the soluble proteins, and centrifuged to clarify. The pellet was further extracted with a second buffer to obtain the less soluble protein fraction. The lysate solution may appear turbid at cold temperatures due to insolubility of buffer components. The solution should clear upon warming to room temperature.

Extraction 1: PBS, pH 7.4 1 ug/ml Aprotinin 1 mM NaF

Modified RIPA Buffer: 1 mM EDTA 1 ug/ml Pepstatin-A 0.1% SDS
0.25% Na deoxycholate 1 ug/ml Leupeptin 1 mM PMSF
1 mM Na₃VO₄

Extraction 2: PBS, pH 7.4, 5.0 M Urea, 2.0 M Thiourea, 50mM DTT, 0.1% SDS