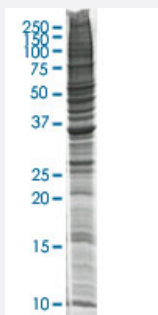


C32 (human amelanotic melanoma) nuclear extract lysate (denatured)

Catalog # L002V3

Size 50 ug

Applications



SDS-PAGE Gel

Specification

Product Description Nuclear extract cell lysate (denatured).

Tissue Skin

Host Human

Preparation Method Nuclear extract was prepared by using a modified protocol of Dignam et al. Cells were Harvested and homogenized in Buffer A, and then centrifugated at 25,000 g for 20 minutes to remove cytoplasm and pellet the nuclei. The pellet was re-suspended in Buffer C, and then the suspensions were centrifuged to collect nuclear extract. The supernatant was dialyzed against Buffer D. The dialysate was then centrifuged, divided into aliquots, and stored at -80°C. The protein concentration was determined by the method of Bradford (Bio-Rad protein assay, microplate standard assay). The lysate was adjusted to 2.5 mg/ml, and then mixed with 5X Sample Buffer to become final 2 mg/ml in 1X Sample Buffer. The lysate was heated at 95°C for 5 min, and cooled rapidly.

Lysis Buffer Buffer A: 10mM HEPES pH 7.9, 1.5mM MgCl₂, 10mM KCl, 0.5 mM DTT.
Buffer C: 20mM HEPES pH 7.9, 25%(v/v) Glycerol , 0.42M NaCl , 1.5mM MgCl₂, 0.2 mM EDTA, 0.5 mM DTT & 0.5 mM PMSF.
Buffer D : 20mM HEPES pH 7.9, 20%(v/v) glycerol, 50mM KCl, 0.2 mM EDTA, 0.5 mM DTT & 0.5 mM PMSF.

Quality Control Testing 12.5% SDS-PAGE Stained with Coomassie Blue.
SDS-PAGE Gel

Recommend Usage	The lysate is ready to load on SDS-PAGE for Western blotting.
Storage Buffer	In ready-to-use 1X Sample Buffer (50 mM Tris-HCl, 2% SDS, 10% glycerol, 300 mM 2-mercaptoethanol, 0.01% Bromophenol blue).
Storage Instruction	Store at -80°C. Aliquot to avoid repeated freezing and thawing.

Applications

- Western Blot