

PLC/PRF/5 (human hepatoma) nuclear extract lysate

Cat. No. PLC-PRF-5-1370H **Lot. No.** (See product label)

SPECIFICATION

Product Overview

Species Human

Tissue Type Liver

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 centrifuged 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.

Size 50 ug

Recommended The lysate is ready to load on SDS-PAGE for Western blotting.

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Usage	
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;

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