Cell nuclear protein preparation protocol for western blot

Isolation of nuclei from cells.

Reagents

Buffer A

20 mM Tris pH 7.5–8.0

100 mM NaCl

300 mM sucrose

3 mM MgCl2

Buffer A contains sucrose and should be kept frozen at -20°C.

Buffer B

20 mM Tris pH 8.0

100 mM NaCl

2 mM EDTA pH 8.0

Keep at 4°C.

Buffer C

20 mM Tris pH 8.0

100 mM NaCl

2 mM EDTA pH 8.0

2% SDS

Keep at room temperature.

Method

Prepare 1 ml of buffer A with added cocktail of usual protease inhibitors from frozen stock and store on ice.

Add 500 µl buffer A per large petri dish on ice and scrape thoroughly. Leave on ice for 10 min.

Centrifuge at 4°C at 3,000 rpm for 10 min.

Remove supernatant and retain. This will contain everything except nuclei.

On ice, resuspend the pellet in 374 µl buffer B and add 26 µl of 4.6 M NaCl to give 300 mM NaCl (high salt helps lyse membranes and forces DNA into solution).

Homogenize with 20 full strokes in Dounce or glass homogenizer on ice.

Leave on ice for 30 min.

Centrifuge at 24,000 g for 20 min at 4°C.

Aliquot supernatant, remove 10 µl for protein quantification and store at -70°C

Origin: Pioneer Biotechnolgy, Inc of China
Distributor: Pioneer Biotechnolgy,Inc
Contact: Tel 029-88237772 or 技术支持电话：13484545009（微信同号） Fax 029-88237772
E-mail : xfyangbio@163.com
Internet: www.xfbio.com