Cell Lysate preparation - general protocol

everest BIOTECH
CREATING NOVEL ANTIBODIES

Datasheets that this protocol applies to – All those where western blots using goat polyclonals on tissue culture cell lysates are described, except where: transfected cell lysates are used; the data is clearly attributed to an external collaborator.

Starting material: Cells from tissue culture, after establishing the cell density. Cells are washed in ice-cold PBS twice and spun at 1400rpm for 3min at 4C.

- De-compact the cell pellet by tapping the tube
- Add RIPA buffer to reach a cell density of 1 x 10^8 cells per ml and mix by vortex
- Keep the mix on ice for 20min while vortexing every 5min.
- Do not exceed the 20min incubation to prevent disintegration of the nuclei
- Divide over 1.5ml tubes and spin at 13000rpm for 3min at 4C
- Reserve 20ul for protein determination by BioRad Protein Determination Kit
- Adjust the lysate to 5mg protein /ml by adding RIPA buffer
- Store aliquots in liquid nitrogen

RIPA buffer: 20mM Tris-HCL pH7.4, 150mM Na\Cl, 1mM EDTA, 1% Triton-X100, 1% sodium deoxycholate, 0.1% SDS with freshly added PMSF to 1mM and with freshly added aprotinin and leupeptin to 5ug/ml just before use.

Last reviewed 24/09/2010