

NATIVE SDS-PAGE/SOD ACTIVE STAIN

Materials/Reagents:

Buffer C

20 mM HEPES pH7.9
25% glycerol
420 mM NaCl
1.5 mM Mg Cl₂
0.2 mM EDTA

10% Native-gel (6 ml/gel)

30% Acrylamide 2ml
TrisHCl pH 8.8 1.5 ml
dH₂O 2.5 ml
10% APS 30 µl
TEMED 3 µl
(do not need stacking gel)

Lysis buffer

10 mM TrisHCl pH 7.4
10 mM NaCl
3 mM MgCl₂
0.5% NP-40 or 0.1% TritonX-100

Running Buffer

Tris Base 3g (25 mM)
Glycine 14.4g (192 mM)
in 1000 ml of dH₂O

Riboflavin-NBT solution

Riboflavin 1 mg
Nitro Blue Tetrazolium (NBT) 2.5 mg
in 10 ml dH₂O

Procedure:

1. Preparation of whole cell extract (WCE):
Add appropriate volume (approximately twice volume of the pellet) of Buffer C + 1 mM PMSF.
Lyse the cell by freeze thaw for Buffer C extraction or add Lysis buffer
After 10 min of incubation on ice, centrifuge 20 min at 4°C, take sup.
Protein assay (about 1-5 µg protein/µl)
2. Load 12 µg of WCE with 50% glycerol and dye on the 10% Native PAGE gel, constant 100 V, ~1 h
3. Soak the gel in the 2-5 ml of Riboflavin-NBT solution. Incubate at R.T. for 15 min in dark*
(*:Riboflavin is light sensitive. Keep the gel in dark room or drawer during incubation)
4. Remove the Riboflavin-NBT soln. Add 2-5 ml of 0.1% TEMED and incubate at R.T. for 15 min in dark.
5. Remove the solution, light induces super oxide synthesis.
6. Dry the gel with keeping light off. (Scan the gel before gel-dry)

Comments:

It takes 15-30 min to develop. Gel color turns into blue-purple and SOD bands are white.