Nuclear Protein Extraction 7/7/96

Buffer A

10 mM HEPES pH7.9 – 100 µl 1 M
10 mM KCl – 100 µl 1 M
1.5 mM $MgCl_2$ – 15 µl 1 M
dH2o Sterile – to 10 mls

Buffer B

20 mM HEPES pH7.9 – 200 µl 1 M
10% glycerol – 1 ml 100%
420 mM NaCl – 840 µl 5 M
1.5 mM $MgCl_2$ – 15 µl 1 M
0.2 mM EDTA – 4 µl 0.5 M
dH2o Sterile – to 10 mls

Buffer C

20 mM HEPES pH7.9 – 200 µl 1 M
30% glycerol – 3 ml 100%
1.5 mM $MgCl_2$ – 15 µl 1 M
0.2 mM EDTA – 4 µl 0.5 M
dH2o Sterile – to 10 mls

Add: 1 M DTT – 50 µl / 10 ml
100 mM PMSF – 50 µl / 10 ml
10 mg/ml Aprotinin – 10 µl / 10 ml
} – to Buffer A, B and C just before use.
– place buffers on ice for extended period of use.

- Collect 100 million cells at 1-2000 rpms 10 mins at 4°C.
- Wash cells once in cold PBS 25 mls. Pellet as above.
- Resuspend in 4 Vols. to Size of the pellet in buffer A = 500 µl – 1000 µl
- Incubate on ice for 1 hour. Transfer to douncer and homogenize 20-30 Strokes
- Transfer to 1.5 mls eppendorf tube. Centrifuge @ 2000 rpms 5 mins at 4°C.
- Aspirate and resuspend in 1 ml Buffer A. Centrifuge @ 2000 rpms 5 mins at 4°C and aspirate
- Resuspend in 2 volumes pellet with Buffer B.
- incubate on ice for 30 mins. Microfuge 20 mins @ 13,000 rpms 4°C.
- transfer to fresh 1.5 ml eppendorf. Add equal volume of Buffer C.
- Snap freeze in dry ice ethanol bath. Store at -80°C

Bradford Assay:

795 µl dH2o
5 µl protein (NE)
200 µl Bradford
1000 µl

} Dilution 1:200

* Need Concentration of Nuclear Extract 3-5 µg / µl

Binding Buffer

20 mM Phosphate buffer pH 6
10 mM $MgCl_2$
0.1 mM EDTA
2 mM DTT
0.01% NP40
0.1 mM PMSF
50-100mM NaCl
15% glycerol
100ng/ul BSA

- * Binding rxns were done in 19µl of binding buffer
- For gel retardation protein fractions were added to binding buffer containing DNA labeled probe (1000 - 5000 cpms) and poly dI-dc as non-specific Competitor

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Protein Samples : Buffer C

20mM potassium phosphate buffer pH 6.0
10mM $MgCl_2$
0.1mM EDTA
1mM DTT
0.01% NP40
0.1mM PMSF
10% glycerol
100 mM NaCl

} protein samples dialyzed and stored in this buffer

- 0.25ng 5'-end labeled oligo in 1µl Rxn Vol. w 200ng poly dI-dc and 3-6µg of Nuclear extract
- with purified protein no poly(dI-dc) but 100µg/ml BSA.

* Incubations : 1 — 15 mins R.T
2 - 30 mins R.T
3.— 1 hour 4°C w antibody before EMSA

For Gel Shift Assay:

Protocol:

Binding Buffer:

20 mM Phosphate buffer pH6.0,
10 mM $MgCl_2$
0.1 mM EDTA Na_2
2 mM DTT
0.01% PMSF
100 mM NaCl
15% Glycerol
100 µg/ml BSA

Dialysis Buffer for purified Protein Samples: Buffer C.

20 mM K PO_4 buffer pH6.0
10 mM $MgCl_2$
0.1 mM EDTA
1 mM DTT
0.01% NP40
0.1 mM PMSF
10% glycerol
100 mM NaCl

* proteins also stored in this buffer

For Gel Retardation Assay:

1. Do rxns in total volume of 20µl of binding buffer
2. Amount of probe equivalent to 0.25ng 5'-end labeled oligo. with 200ng poly dI-dC in 10µl buffer.
3. Amount of protein in total rxn 3-6µg
4. When using purified protein no poly dI-dC but 100µg/ml BSA
5. Incubate rxns for 30 mins at R-Temp
6. For rxns which includes antibodies incubate for 1 hour at 4°C prior to running EMSA gel.