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SDS-PAGE/Western Blot protocol

Reagents

1) **10X SDS buffer** (500 ml of 0.25M Tris, 1.92 M glycine, 1% SDS)
   Add to ~300 ml of dH_2O: 15.15 g Tris Base
   72 g Glycine
   5 g SDS (wear face mask when working with SDS)
   Fill up to 500 ml w/ dH_2O
2) **10X Transfer buffer for semi-dry transfers w/o methanol** (500 ml of .25M Tris, 1.92 M glycine)
   Add to ~300 ml of dH_2O: 15.15 g Tris Base
   72 g Glycine
   Fill up to 500 ml w/ dH_2O
3) **1X Transfer buffer for semi-dry transfers w/ methanol** (500 ml)
   Add: 50 ml of 10X Transfer buffer w/o methanol
   150 ml of 100 % methanol
   Fill up to 500 ml w/ dH_2O
4) **10X Transfer buffer for wet transfers w/o methanol** (500 ml of 0.48M Tris, 0.39 M glycine, 0.4% SDS)
   Add to ~300 ml of dH_2O: 29.07 g Tris Base
   14.64 g Glycine
   2 g SDS
   Fill up to 500 ml w/ dH_2O
5) **1X Transfer buffer for wet transfers w methanol** (500 ml)
   Add: 50 ml of 10X Transfer buffer w/o methanol
   100 ml of 100 % methanol
   Fill up to 500 ml w/ dH_2O
6) **Blocking buffer** (500 ml of 50 mM tris pH 7.5, 150 mM NaCl, 0.05 % NP-40, 0.25 % gelatin)
   Add: 25 ml of 1M Tris pH 7.4
   15 ml of 5M NaCl
   250 ul of NP-40
   1.25 g gelatin
   Fill up to 500 ml w/ dH_2O; autoclave and store at 4C
7) **TBSt** (30 mM Tris pH 7.4, 300 mM NaCl, 0.2% tween 20)
   Add: 15 ml of 1M Tris pH 7.4
   30 ml of 5M NaCl
   10 ml of 10% tween 20
   Fill up to 500 ml w/ dH_2O
8) **Stripping buffer** (0.2M Glycine, 0.05% tween 20 pH 2.5)
   Add to ~300 ml of dH_2O: 7.5 g of glycine
   2.5 ml of 10% tween 20
   Fill up to 500 ml w/ dH_2O

Procedure

1) Load 20-30 ug of proteins per sample onto a 10 % SDS-PAGE gel. Run in 1X SDS buffer at 120 V for 1.5 - 2 hours
   a. Also load 15 ul of SpectraBR (Fermentas, Glen Burnie, Maryland) protein ladder
2) Pre-treat PVDF membrane w/ 100% MeOH for 1 min at room T
3) Equilibrate gel, filter papers, and PVDF membrane in 1X Transfer buffer w/ methanol for 2-3 minutes at room T
4) Transfer proteins onto 0.2 um PVDF membrane
   a. for 45 minutes at 25V (Semi-dry transfer, Bio-Rad apparatus) at room T
   b. For 1 hour at 100 V (wet transfer) at 4C
5) Block for 1 hr at room T in 5% milk in TBSt
6) Incubate in primary antibody in 5% milk + TBSt
   a. Generally, do overnight incubation at 4C (cold room) on a shaker; for Actin can incubate at room T for 1 hour
   b. See antibody data sheet for suggested antibody concentration
7) Wash 3x 10 minutes with TBSt
8) Incubate in secondary antibody in 5% milk + TBSt for 1 hour at room T
   a. Generally use 1:1000 dilution of secondary antibody; for Actin, can use 1:2000
9) Wash 3x 10 minutes with TBSt
10) Detect with ECL substrate for 5 minutes

Note: Use GE/Amersham ECL or Pierce ECL for good antibodies; for antibodies that result in weak signals use Supersignal Pierce West Femto ECL

11) Expose blot to film

Note: Time of exposure will depend on signal strength

12) To strip blot (Use microwavable container!!!):
    a. Rinse membrane in TBSt
    b. Add enough stripping buffer to cover the membrane
    c. Microwave on high for 1 min
    d. Nutate (rock) on shaker for 30 min at room T
    e. Rinse in TBSt for 5 min
    f. go to Step 4…and reprobe with other antibody