1. Application field

Buffers for protein transfer and western-blotting

2. Referent persons

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3. Heath and safety :

Methanol :

GHS02, GHS06, GHS08

Hazard statements
H225-H301 + H311 + H331-H370
Personal Protective Equipment
Eyeshields, Faceshields, full-face respirator (US), Gloves,

Thimerosal

GHS06, GHS08, GHS09

Hazard statements
H300 + H310 + H330-H373-H410
Personal Protective Equipment
Eyeshields, Faceshields, full-face particle respirator type N100 (US), Gloves,

-Manipulate buffers with methanol under fume hood.

-Throw away effluents with methanol in specific waste « Effluents aqueux proteines + HPLC » .

4. Buffers :

Buffers for Protein transfer:

Depending on the aim and timing, the different buffers below can be used for protein blotting

1) Semi-dry blotting

glycine 39 mM 2,92 g

Tris 48 mM 5,81 g
SDS 0,0375 % 1,875 ml of 20 % solution
methanol 20 % 200 ml
H_2O qsp 1 l
transfer at 0,8 mM/cm2 for 1h 15 or
transfer at 12 V for 15 min , and 24 V for 45 min

2) liquid transfer in CAPS-methanol

Caps 10 mM
methanol 10 % 100 ml
adjust to pH 11 with NaOH 6N
transfer 35 V for 16 h at 4°C

3) Liquid Tris-Glycin-methanol

Tris 10 mM 1.82 g /1.5l
Glycin 100 mM 11.26 g/1.5l
Methanol 10 % 150 ml
H_2O qsp 1.5 l
Transfer 70 v for 1H30 at 4°C

4) Liquid Tris-borate (for sequencing)

- equilibrate gels for 2 h in boric acid 50 mM, SDS 0,1 %
  Adjust to pH 8 with NaOH 6N

- transfer in boric acid 50 mM
  Tris 50 mM
  35 V for 16 h at 4°C

Buffers for Western

PBS 10X buffer

KH_2PO_4 40 mM
Na_2HPO_4 160 mM
NaCl 1,15 M
Adjust pH to 7,3 with NaOH 6N
Add thimerosal 0,002 % (p/v) for long conservation

PBSTB buffer

	finale conc	quantity
PBS	1X	50 ml of 10 X buffer
Tween-20	0,1 %	500 mg
BSA	1 %	5 g
H2O qsp		500 ml

Buffer volumes : 40 ml for small membrane in square petri dish and 80 ml for big membrane;
Possible to incubate primary Antibody in a sealed bag.

5. Operating mode for Western-blot :

After blotting, wash the PVDF membrane in PBS

Membrane saturation :

- **In PBSTB**
  1 h at room temperature or ON at 4°C

- **Primary antibody**
  Dilute in PBSTB, incubate 2 h at room temperature or ON at 4°C

- **Wash** with PBSTB 2 x 10 min

- **Secondary antibody: peroxidase conjugate**
  dilute 20.000 X in PBSTB, incubate 1 h at room temperature

- **Wash** with PBS 4 x 5 min

- **revelation: chemiluminescence**
  Drain the membrane on a paper
  Incubate the membrane in presence of a mix of substrates "Super signal West Pico Chemiluminescent substrate" (Thermo Sci.):
  x ml solution A + x ml solution B during 5 min
  Drain the membrane on a paper

Expose and reveal using LAS 3000.