Solutions for immunocytochemistry

1- PBS (0.05M):

250 ml of 0.2 M phosphate buffer pH 7.4 + 750 ml dd water + 9 g NaCl.

2- TPBS:

10 mM Tris + 0.05% thimoresal (Sigma #T-5125) + 10 mM phosphate buffer pH 7.4 + 0.9% NaCl. When using this buffer, incubations can be done at room temperature. **Do not use containers with aluminum or other metals, TPBS will react with them**.

To make 7.5 L (fills one container)	
350 ml phosphate buffer 0.2 M	700 ml phosphate buffer 0.2 M
3.75g Thimoresal	Thimoresal 7.5 g
9.08 g Tris (BASE)	Tris 18.16 g (BASE)
67.5 gSodium chloride(0.9%)	Sodium chloride 135 g (0.9%)
Adjust pH to pH 7.4 with 10 N HCl	Adjust pH to pH 7.4 with 10 N HCl
Add dd H_20 to 7.5 L	Add dd H_20 to 15 L

3- PBS or TPBS with 1% NGS (Normal Goat Serum) + 0.3% Triton X-100:

1 L of PBS or TPBS
10 ml of NGS
3 ml of Triton

Put a stir-bar and stir for 10 min

Because it contains NGS, this solution as to be kept at 4º C and should be reprepared after 5 days.

4- PBS or TPBS with 0.3% Triton X-100:

1L of PBS or TPBS
3 ml of Triton

Put a stir-bar and stir for 10 min

5- Block:

3% NGS:
50 ml of solution #3 (above) + 1 ml of NGS

10% NGS:
40 ml of solution #3 (above) + 4 ml of NGS

6- GAR stock: 1 ml dd H_2O + 1.5mg biotinylated goat anti-rabbit IgG (Vector)

7- 30% sucrose: 200 ml of dd H_2O + 200 ml TPBS + 150 g sucrose

8- 0.05 M Tris Buffer: 6.06g. Trizma HCl + 1.39g. Trizma Base + 1 Liter DD H_2O.

PH 7.6 at 25^0 C.

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PHOSPHATE BUFFER

Final solution: 4 liters of 0.2 M $NaP0_4$, pH 7.6

Ingredients: 92 g $Na_2.HP0_4$ (dibasic)
21g $Na.H_2P0_4.H_20$ (monobasic)
4 liters of double distilled water (dd H_20)

Start with 1 or 2 liters of dd H_20 and add the Na_2HP0_4 and the NaH_2P0_4, agitate until completely dissolved. Filter into bottle and add dd H_20 until final volume is 4 liters.

BAXTER: 1-800-848-4744 (USA)
- Sodium phosphate dibasic anhydrous:
  500 g (catalogue #7917-500*NY)
- Sodium phosphate monobasic anhydrous:
  500 g (catalogue #7892-500*NY)
- Whatman quality grade filters, pre-pleated, quality circle:
  15.0 cm diameter: Catalogue #09-832L
  18.5 cm diameter: Catalogue #09-832M
  (100 filters per package)

PARAFORMALDEHYDE

Final solution: 4 liters of 8% paraformaldehyde, pH 7.6

Ingredients: 360 g of paraformaldehyde powder
10 N NaOH
4 liters of double distilled water (dd H_20)

All procedures are done in a well ventilated hood! Heat 3 liters of dd H_20 to 55 to 60^oC. Never let the temperature rise to more than 65^oC, it will ruin the

paraformaldehyde; when this happens, the solution stays cloudy and you should start over again. Transfer the flask to heating-stirring plate and set temperature to 60ºC. Add 360 g of paraformaldehyde powder while stirring. Check temperature frequently to make sure it does no go over 65ºC. After 1 hour of stirring add 10 to 15 drops of Na0H to solution. Let solution stir until it becomes clear (if it is cloudy, it is not ready!). Then gravity filter, using a paper filter and a funnel, into 4 liter bottle. Add dd H_20 until final volume is 4 liters.

ELECTRON MICROSCOPY SCIENCE. Tel. 1-800-523-3874.
Paraformaldehyde EM grade (500g) Catalogue # 19200

BAXTER
Sodium hydroxide (NaOH) 10.0 NORMAL (volumetric solution)
1 liter Catalogue # H385-1*NY

30% SUCROSE SOLUTION

BAXTER
400 ml of dd H_20 + 400 ml of 0.2 M phosphate buffer + 300 g sucrose
Sucrose crystals!: Catalogue #8360-2.5*NY

FORMALINE 5%

SIGMA
We need a few ml of the concentrated solution (37%) that we will dilute with phosphate buffer.