

## **IMMUNOCYTOCHEMISTRY FOR NEURON CULTURES**

### **1. FIXATION AND PERMEABILIZATION**

#### 10X PBS STOCK

82 g NaCl

4.3 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O

17.4 g Na<sub>2</sub>HPO<sub>4</sub>

Fill to 1L with dH<sub>2</sub>O

Use at 1X

Fixatives are best prepared fresh (should be less than one week old) and stored at 4°C.

We commonly use the following fixatives:

#### A. 4% paraformaldehyde/4% sucrose in PBS

Preparation:

Heat to 60°C:

60mL dH<sub>2</sub>O

4g paraformaldehyde

When at 55-60°, add 1-2 drops of 1N NaOH, stir until solution is clear and paraformaldehyde is dissolved. Do not allow temperature to rise above 60°.

Then add 10ml of 10X PBS (see below) and 4g sucrose.

Add dH<sub>2</sub>O to yield 100ml.

Use:

Warm fix to 37°C.

Place cells into fix for 15-30 min. (You may wish to keep warm by putting the cells/fix backing an incubator/oven at 37°C, although often we just warm the fix for 20 min. in a water bath (set to 37°C) and fix the cells on our benchtop).

Rinse in PBS (2X, 1' ea.).

Permeabilize in 0.15-0.3% Triton X-100 in PBS, 5-10 min.

Rinse 3X, 5' PBS.

Proceed to immuno steps, or 1-4 days in PBS at 4°C.

### N2 Supplement

1.0 ml Insulin stock (final concentration 5 µg/ml)

1.0 ml Progesterone stock (final concentration 20 nM)

1.0 ml Putrescine stock (final conc. 100 µM )

1.0 ml Selenium dioxide stock (final conc. 30 nM)

100.0 mg transferrin (Sigma\* #T2252)

Filter sterilize. Store frozen in 10 ml aliquots for up to 1 month.

Insulin stock (1000X):

50.0 mg insulin (Sigma\* #I5500)

10.0 ml 0.01 N HCL (1 N HCL = 8.6ml conc. HCL per 100 ml water; then dilute this 1:100)

Progesterone stock (1000x)

63.0mg progesterone (Sigma\* #PO1301; MW 314.5)

100.0ml absolute ethanol

This gives a 100,000 X stock. Dilute this 1:100 with water to make the 1000X stock solution.

Putrescine stock (1000X):

161.0mg putrescine (Sigma\* #7505; NW 161.1)

10.0 ml water.

Selenium dioxide stock

33.0mg selenium dioxide (Sigma\* #S9379; FW 110)

100.0ml water

This gives a 100,000X stock. Dilute this

1:100 with water to make the 1000X stock solution.

These 1000X stocks can be stored frozen in 1 ml aliquots.

Ovalbumin (1%)

1.0g ovalbumin (Sigma\* #A-5503)

100.0 ml MEM

Add to medium and allow to sit without mixing to dissolve (1/2 hour); albumin will denature if stirred. Filter through a prefilter (eg. Nalgene\* 281-5000) and a 2.0 um sterilizing filter. Store frozen in 10 ml aliquots.

**MEDIA (rev.1/92)**

**MEM\***

500.0 ml 1X MEM (Gibco\* #11095-23)

15.0 ml glucose (20%)

5.0ml pyruvate (100X)

**MEM + 10% Horse Serum (plating medium)**

90.0 ml MEM\*

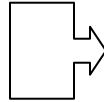
10.0 ml horse serum (JRH Biosciences\*#12-449-78P)

**N2.1**

80.0 ml MEM\*

10.0 ml N2 supplement

10.0 ml ovalbumin (1%)



NEUMANS

**Glial MEM**

500.0 ml MEM\*

50.0 horse serum (JRH Biosciences\* #12-449-78P)

5.0 ml penicillin/streptomycin (100X)

**BSS (Ca-Mg free)**

10.0 ml 10X BSS (Hank's CMF – Gibco\* # 14180-012)

1.0 ml HEPES (1M) Gibco # 15630-080)

1.0 ml penicillin/streptomycin (100X)

88.0 ml water (MilliQ)