APPENDIX 1

SOLUTIONS

Phosphate Buffer Saline (PBS)

NaCl	9 g
0.4M phosphate buffer, ph 7.4	250 ml
distilled water	650 ml

pH was adjusted to 7.4 with NaOH.

The solution was made up to 1 litre with distilled water.

Tris-Phosphate Buffer Saline (TPBS)

Trizma Base	4.844 g
0.4M Phosphate buffer	100 ml
Merthiolate	2 g
NaCl	36 g

pH was adjusted to 7.4 with HCl

The solution was made up to 4 litres with distilled water.

4% formaldehyde

0.4M phosphate buffer	250 ml
40% Formaldehyde (Ajax FineChem Pty Ltd, Australia)	100 ml

pH was adjusted to 7.4 using NaOH

The solution was made up to 1 litre with distilled water.

Durcupan (Resin)

Part A (Epoxy Resin)	10 ml
Part B (Harderner)	10 ml
Part C (Accelerator 960)	0.3 ml
Part D (dibutlyphthalate)	0.3 ml

The solution was vigorously mixed for 1 to 2 minutes.

Calcium Free Krebs solution

Double strength Ca ++ free Krebs solution.

NaCl	26.88 g
KCl	1.4 g
Na_2HPO_4	0.6 g
$NaHCO_3$	8.4 g
$MgCl_2$ and	4.8 g
D-glucose	8 g

The solution was made up to 2 litres with distilled water.

Single strength Ca ++ Krebs solution was prepared by dilution with distilled water.

Pre-incubation solution: Nickel-intensified Diaminobenzidine (Ni-DAB) reaction

0.4M sodium phosphate buffer, pH 7.4	10 ml
0.4% NH_4Cl	400 µl
20% glucose	400 µl
Distilled water	27.6 ml
50 mg/ml DAB	400 µl

1% Nickel Ammonium Sulfate	1.6 ml

Nickel-intensified Diaminobenzidine (Ni-DAB) reaction Reaction mix

Pre-incubation solution	1 ml
Glucose oxidase (Sigma Aldrich, USA)	2 µl

0.4M phosphate buffer

Na_2HPO_4	45.42 g
NaH_2PO_4	12.48 g

The reagents were dissolved in 900 ml of distilled water

pH was adjusted to 7.4 with NaOH

The solution was made up to 1 litre in distilled water.

0.1M phosphate buffer

0.4 M phosphate buffer, pH 7.4	250 ml
Distilled water	750 ml

Immunobuffer (IB)

Triton X-100 (Chem-Supply, Australia)	3.3 ml
0.4M phosphate buffer	1 litre

0.05% Sodium azide in 0.1M phosphate buffer

0.4M phosphate buffer, ppH7.4	100 ml
200× Na azide	2 ml

The solution was made up to 400ml with distilled water.

30% methanol 1% hydrogen peroxide

Absolute methanol (Ajax Finechem Pty Ltd, Australia)	30 ml
Hydrogen peroxide (Chem-Supply, Australia)	3.3 ml

The solution was made up to 100ml with distilled water.

10% normal horse serum – immunobuffer (10% NHS-IB)

Normal horse serum (Gibco, USA)	5 ml
Immunobuffer	45 ml

1% normal horse serum – immunobuffer (1% NHS-IB)

Normal horse serum (Gibco, USA)	0.5 ml
Immunobuffer	49.5 ml