APPENDIX B

Media for hybridoma culture

1. Basic RPMI 1640 medium (per 1 liter)

The medium consisted of the following ingredients:

RPMI-1640 powder	10.40	g
HEPES, MW 238.31	5.9525	g
NaHCO ₃	2.016	g
D-glucose	3.60	g
Sodium pyruvate	1.1005	g and
and L-glutamine	0.2923	g

All ingredients were dissolved in 1,000 ml of UDW. Penicillin G, streptomycin and kanamycin were added to the final concentrations of 20,000 units, 200 mg and 200 mg per liter of the basic RPMI medium, respectively. The medium was sterilized by filtering through a sterile Millipore membrane (pore size $0.22~\mu m$), aliquoted and kept in sterile bottles at -20°C.

2. Complete RPMI 1640 medium

The medium consisted of the following ingredients:

Sterile, heat-inactivated fetal calf serum	10.0	ml
(Starrate Co., Australia)		
Basic RPMI 1640 medium	90.0	ml

3. Trypan blue solution (0.3%)

Trypan blue (60 mg) was dissolved in 20 ml of normal saline solution (NSS). The solution was sterilized by filtering through a sterile Millipore membrane (0.45 μ m) and kept at 4°C until use.

4. CD hybridoma medium

Commercial CD hybridoma medium (Gibco, USA) was supplemented with 4% L-glutamine (Gibco).