APPENDIX C

Media and reagents for hybridoma/monoclonal antibody production

1. Basic RPMI medium (serum free-RPMI)

The medium consisted of the following ingredients:

RPMI 1640 (Gibco Co., U.S.A)	10.40 g,
HEPES, MW 238.31 (Sigma Chemical Co., St Louis, Minnesota, USA)	5.95 g,
$NaHCO_3$	2.02 g,
D-glucose	3.60 g,
Sodium pyruvate (Sigma Chemical Co.)	1.10 g,
L-glutamine (Sigma Chemical Co.)	0.30 g
and UDW to make 1,000 ml	10.40 g,

The solutions of penicillin G, streptomycin and kanamycin were added to the final concentration of 20,000 units, 200 mg and 200 mg per liter, respectively. The medium was sterilized by filtering through a sterile Millipore membrane (pore size 0.22 μm). The medium was kept in aliquots of 100 ml in steriled bottles at -20°C.

2. Complete RPMI medium

2.1 Complete RPMI medium (10% FBS, v/v)

The medium consisted of the following ingredients:

Sterile, heat-inactivated fetal bovine serum (PAA Laboratories GMbH, Austria)	10.0 ml
and basic RPMI medium	90.0 ml

2.2 Complete RPMI medium (20% FBS, v/v)

The medium consisted of the following ingredients:

Sterile, heat-inactivated fetal bovine serum (PAA Laboratories GMbH, Austria)	20.0 ml
and basic RPMI medium	80.0 ml

3. Stock solutions

3.1 Azaguanine (2.0 mM)

The solution was prepared by dissolving 30 mg of 8-azaguanine (Sigma Chemical Co., St Louis, Minnesota, USA) in UDW by dropwise adding of 1 N NaOH until the reagent was completely dissolved. The pH of the solution was adjusted to 7.0 with 1 N HCl and then the volume was made up to 100 ml with the UDW. The

preparation was sterilized by filtering through a sterile Millipore membrane, aliquoted in 5 ml volume and stored at –20°C.

3.2 Hypoxanthine (5.0 mM)

The solution was prepared by dissolving 68 mg of hypoxanthine (Sigma Chemical Co.) in 100 ml of UDW at 50°C and sterilized by Millipore membrane filtration. The preparation was aliquoted in 5 ml volume and stored at –20°C.

3.3 Azaserine (1.0 mM)

The solution was prepared by dissolving 17.31 mg of azaserine (Sigma Chemical Co.) in 100 ml of UDW. The solution was sterilized by Millipore membrane filtration and stored at –20°C in 5 ml aliquots.

4. Myeloma cell culture medium (H-AZA medium)

The medium was prepared by diluting 1 ml of stock solution of 8-azaguanine in 100 ml of complete RPMI-1640 medium.

5. Hybridoma selective medium (HA medium)

The medium was prepared by mixing 1 ml of stock solution of hypoxanthine, 1 ml of stock solution of azaserine and 100 ml of complete RPMI-1640 medium together.

Selectivity of this medium was tested against myeloma cells. Growth of myeloma cell was inhibited and signs of death appeared within 2-3 days.

6. H medium

The medium contained 1 ml of stock solution of hypoxanthine and 100 ml of complete RPMI-1640 medium.

7. Fusogen (polyethylene glycol solution; 50% w/v solution)

The solution was prepared on the day of cell fusion by melting 1 g of polyethylene glycol (PEG) 4,000 (Sigma Chemical Co.) in 2 ml of NSS by boiling for 30 min. It was kept in a 37°C water bath until use. Freshly prepared PEG was used in each cell fusion.

8. Freezing medium

This medium was prepared immediately before use. Nine parts of BS were mixed with one part of dimethylsulfoxide (DMSO) and the mixture was kept at 4°C.