APPENDIX 7

MONOCLONAL ANTIBODY PRODUCTION (REAGENTS USED)

Complete culture medium
Dulbecco modified Eagle medium (DMEM) having high-glucose formula (4.5 g glucose/liter; GIBCO No.430-2100) was supplemented to the indicated concentrations with the following additives:

- 2.8 g/liter Sodium bicarbonate (33.3 mM)
- 4.8 g/liter HEPES (20 mM)
- 10% Fetal calf serum (vol/vol)
- 10 ml/liter L-glutamine (2 mM)
- 10 ml/liter Sodium pyruvate (1 mM)
- 10 ml/liter Penicillin (50 IU/ml) and streptomycin (50 μg/ml)

Supplemented DMEM
Dulbecco modified Eagle medium (DMEM) (GIBCO NO.430-2100) was supplemented with the following:

- 2.8 g/liter sodium bicarbonate (33.3 mM)
- 4.8 g/liter HEPES (20 mM)

100 x HT Solution
To a water of 250 ml, 340.3 mg of hypoxanthine and 96.9 mg of thymidine was added. The mixture heated to 70°C and was filter sterilized and stored at -20°C.

100 x aminopterin solution
To a 60 ml of water, 17.6 mg of aminopterin was added and dissolved by adding 0.1 M NaOH dropwise. The solution was titrated with HCl to obtain a pH of approximately 8.5. The volume was adjusted to 100 ml and was filter sterilized.
Working solutions were made by diluting to 100 x using complete culture medium. Aliquots were stored at -20°C.

**HAT (hypoxanthine/aminopterin/thymidine) medium**

Complete culture medium was supplemented to the indicated concentrations with the following additives:

- 20% (vol/vol) fetal calf serum
- 0.1 mM nonessential amino acids
- 100 mM hypoxanthine
- 0.4 mM aminopterin
- 16 mM thymidine

**HT Medium**

Prepared similar to HAT medium but without aminopterin solution.

**Sucrose solution (0.34 M)**

- 58.2 g Sucrose
- H₂O to 500 ml
- Filter sterilized and stored at 4°C

**PEG (Polyethylene glycol) solution**

To a 100 ml glass bottle, 10 g of PEG 4000 (Merck) was added and autoclaved for 10 min at 121°C. On cooling the molten PEG to 50°C, the prewarmed supplemented DMEM containing 5% (vol/vol) dimethylsulfoxide (DMSO), was mixed and distributed into glass bottles (3 ml capacity). The PEG solution was stored at 4°C in dark.