CELL HYBRIDIZATION USING PEG

A) Animal Immunization: 1 or 2 injections spaced 2-4 weeks apart, then 1 month to 4 months later a final injection i.v. in the tail vein 3 days before the hybridization experiment.

B) Myeloma Cells: P3Ag8.6.5.3 or NS-O (nonproducers) or NS-I (P3-NSI/1-Ag4-1) (K chain producer) cells, 2 to 10 x 10^7, in log growth.

C) Other Materials:

(We currently use RPMI 1640 rather than DME)

1) 50% w/w PEG:autoclave 10g BDH polyethyleneglycol M.W. 1500. Allow to cool some, but before solidification add 10 ml DME, + gln, - P/S, - pyruvate. After cooling, adjust pH to 7.6-7.8 by allowing CO_2 to come off until color is raspberry red. Warm to 37° for use in fusions. Store at 4° for 2 months.
2) 100 ml DME + gln, - pyruvate, on ice.
3) 200 ml DME + gln, - pyruvate, r.t.
4) 20 ml DME + gln, - pyruvate, 37°.
5) 50 ml DME + gln + 10 units/ml heparin, 4°.
6) 50 ml DME + gln, + gentamycin + pyruvate + 20% heat inact. FCS, r.t.
7) 500 ml DME + gln + gentamycin + pyruvate + 20% h.i. FCS, + HAT, r.t.

D) Procedure: (all washes at 200 x g x 5 min. The fusion is done in the absence of serum).

1) Remove resident peritoneal cells for use as feeder cells by intraperitoneal lavage of 30 ml/rat or 10 ml/mouse DME + 10 units/ml heparin.

   Remove spleen by blunt dissection, cut longitudinally, and tease out cells into ice-cold DME. Place in 10 ml tube. Allow debris to settle out for 5 min on ice and wash cells at 4° in 50 ml DME, suspend in 10 ml ice-cold DME and count viable white cells.
2) Wash myeloma cells at 10° in 50 ml DME and suspend in 10 ml of r.t. DME and count.
3) Mix spleen and myeloma cells in a 4:1 ratio, make total volume to 50 ml DME and wash at 10°.
4) Aspirate all supernatant and suspend pellet by flicking with finger. Place the tube in a beaker containing 37° water and add gradually over 1/2 min 1 ml 50% w/w PEG at 37° while stirring

the pellet with the tip of the 1 ml pipette. Allow to stand 1-1/2 min at 37° with occasional stirring. Dilute slowly with 5 ml DME at 37° over a 3 min period while stirring. Then add 14 ml of DME at 37° over 1 min period. Add 30 ml 20% FCS-DME, centrifuge at 20° and suspend to 1.5 x 10^6 input spleen cells/ml in 20% FCS-DME-HAT. Add normal rat or mouse peritoneal cell feeders (same stain as spleen cell donor) at 2.5 x 10^4/ml. Place 0.2 ml aliquots in Costar 96 well plates (measure out 20 ml/plate) (For one rat spleen about 3 x 10^8 cells, suspend to about 200 ml and place in 10 x 96 well plates).

E) Feeding schedule:

Day 0: hybridization

Days 3,6,9: replace 1/2 - 2/3 medium with HAT-DME + 20% FCS (fewer feedings can be done in this period if medium is not turning yellow, but feed at least every 7 days).

Days 12-28: feed as required (every 2-7 days) with HT-DME + 20% FCS. When wells begin turning yellow within 2 days, all should be fed every 2 days (waiting 3 days will kill cells).

Day 30 on: feed with DME + 20% FCS.

HAT stock solutions:

HT 100 x: hypoxanthine 136 mg
Thymidine 38.8 mg
D.D.W. 100 ml
dissolve at 70°, filter sterilize, and store frozen at -20° (~6 months).

Aminopterin 1,000 x: aminopterin 3.5 mg
0.1N NaOH 1 ml
D.D.W. 19 ml
filter sterilize, store in dark at -20°C (2 months).

HAT 1x: HT 100 x: 5ml
Aminopterin 1,000 x: 0.5 ml
DME + 20% FCS: 500 ml

HT 1x: as above, omit aminopterin.

8-Azaguanine 100x: 8-azaguanine 2.5 mg
0.01N NaOH 10 ml
filter sterilize, store 1 ml aliquots at -20°.

Growth of cells in DMEM and high glucose and pyruvate + 6-8 mM glutamine gives 4x higher MAb yield than RPMI 1640.