

GPT SELECTION:

Reference: Mulligan, RC & P Berg, PNAS (1981) 78, pp 2072-76.

Media per 500 ml bottle:

DME 403 mls (remove 35 mls)
10% Dialyzed FCS 50 mls
PCN/Strp 5 mls
25X Xanthine 20 mls
100X Glutamine 5 mls
50X HAT 10 mls
MPA 5 mls
Aminopterin 1 ml
Thymidine 1 ml

Note: for initial 293 selection, use double MPA (50 µg/ml), after initial selection and for 3T3 selection 25 µg/ml is fine.

REAGENTS:

Dialyzed fetal bovine serum: JRH R-10578, \$210
Xanthine: Sigma X-2001, 5 g, \$20.95
Mycophenolic Acid: Gibco 860-1814IH, 500 mg, \$147
Aminopterin: Sigma A3411, 25 mg, \$43
Thymidine: Sigma T1895, 1 g, \$10.20
50X HAT Supplement; Sigma H-0262, 50 vials, \$129

312.5 mg / 50 mls NaOH
0.5 ml 10M +
49.5 ml milli.Q

STOCKS:

25X XANTHINE: 6.25 mg/ml; add 1.56 g to 250 ml 0.1N NaOH; filter and store at 4 degrees.

0.1 N HCl
100 µl conc + 10 ml
milli.Q
0.1 N NaOH
100 µl 10N + 10 ml
milli.Q

100X MYCOPHENOLIC ACID: 2.5 mg/ml; add 0.25 g to 100 mls 0.1 N NaOH, neutralize with 0.1 N HCl (check with pH paper), filter and freeze in 5 ml aliquots.

25 mg / 10 mls

50X HAT: dissolve in 10 mls H₂O, filter and use immediately or freeze.

+ 10 mls H₂O

500X Aminopterin: 1 mg/ml; add 0.05 g to 50 mls DME, filter and freeze.

10 mg / 10 ml DME

500X Thymidine: 3 mg/ml; add 0.15 g to 50 mls DME, filter and freeze.

30 mg / 10 ml DME