

Equipment and Materials:

ES Medium: (500 ml)

400 ml DMEM

(Gibco Cat. No. 12800

supplemented with 1.2 gm/L NaHCO₃, 6.24 gm/L HEPES)

75 ml fetal calf serum (heat inactivated, 56°C, 30 min.)

(Hyclone Cat. No. A-1115-L, use a lot # that works well with ES cells)

5 ml non-essential amino acids (100x stock, Gibco Cat. No. 11140)

5 ml antibiotics (100x Penicillin Streptomycin stock, Gibco Cat. No. 15070)

5 ml Glutamax

4 µl 2-mercaptoethanol (easier to use 0.4ml of a 1:100 dilution)

-300 ul in 29.7 ml sterile water for dilution.

Feeder Medium: Same as ES medium but with 10% FCS

437 ml DMEM

50 ml fetal calf serum

rest is same as ES media

Hank's balanced salt solution (HBSS):

Gibco Cat. No. 21250

Gelatin solution:

0.2% gelatin in water, autoclave, store at room temperature.

-can be filter sterilized.

Trypsin-EDTA:

0.25% Trypsin (from 2.5% stock, mycoplasma screened, Gibco Cat. No. 15090)

1 mM EDTA in HBSS, filter sterilize and store at -20°C.

90 ml HBSS

10 ml 2.5% Trypsin

200 ul .5M EDTA

-aliquot into 10 ml tubes after filtering.

Freezing solution 2X: (100 ml)

60 ml DMEM (ice cold)

20 ml DMSO

20 ml FCS

Aliquot and store at -20°C

ZAP Buffer:

20 mM HEPES ph 7.0

137 mM NaCl

5 mM KCl

0.7 mM Na₂HPO₄

6 mM Dextrose

0.1 mM 2-mercaptoethanol

Prepare as a 10X stock without 2-mercaptoethanol. To prepare a working solution, dilute 1 ml 10X with 9 ml water, then add 7µl of a 1:100 dilution of 2-mercaptoethanol.

Drug containing medium:

G418: (Gibco, Cat. No. 860-18111J) Prepare a 200 mg/ml active form stock solution in HBSS, aliquot and store at -20°C (will be 1000X). Each new bottle will vary as to amount of active material. (Ex. If 770ug on bottle, $200/770 = 259.7$ mg or .2597 g powder per ml HBSS)

Use at a final concentration of in ES medium of 0.20 mg/ml of active form. (Ex. 500 ul in 500 ml).

Gancyclovir: (Syntex, Cat. No. 00033-2903-48) prepare a 2 mM stock solution in HBSS, aliquot and store at -20°C. Use at a final concentration of in ES medium of 2µM.

Mineral oil:

Filter sterilized

DNA Lysis Buffer:

100 mM Tris-HCl pH 8.5	12.11 g
5 mM EDTA	1.861 g
0.2% SDS	2 g
200 mM NaCl	11.69 g

No need to autoclave.

Proteinase K:

20 mg/ml stock prepared in water, frozen in aliquots at -20°C

TE:

10 mM Tris pH 8.0
1 mM EDTA
For 10 X stock - 100 mM Tris 12.11 g
- 10 mM EDTA 3.722 g
Use Barnstead water. Mix in 1L graduated cylinder.

LIF (leukemia inhibiting factor): If you wish to spend \$100/10⁶ units of LIF and use 500-1,000 units per ml of ES medium, it won't hurt. But if your feeders and serum are good, that is COMPLETELY un-necessary. Germ line transmission of ES cells has been achieved over and over again by groups that NEVER use LIF.

Scalpels and other dissecting tools

Tissue culture plastics:

T25, T75, T175 flasks
10 cm dishes
12, 24, 96 well dishes

Pipetmen: P20, P200 with racked tips

Electroporation apparatus: e.g. Biorad Gene Pulser II

Electroporation cuvettes: 0.4 cm gap (Biorad Cat. No. 165-2088)

12 place pipetor (optional)

Microscope in a tissue culture hood or laminar flow bench

Gamma radiation source

Clinical centrifuge (need not be refrigerated)

Sealable bags and a heat sealer