

MATERIALS

1. Media and solutions

CM (complete medium)

1 x GMEM	Gibco/invitrogen 21710-025	500 ml
Sodium Pyruvate 100 mM	Gibco/invitrogen 11360-039	5 ml
Non-essential amino acids 100 x	Gibco/invitrogen 11140-035	5 ml
Fetal Calf Serum (FCS is tested for optimal growth of ES cells)		50 ml

CM + β + LIF

CM		100 ml
β -Mercaptoethanol (1000x)		0.1 ml
LIF (100x)		1 ml

100% BRL medium

CM conditioned on a monolayer of Buffalo Rat Liver (BRL) cells

60% BRL + β + LIF (60% medium)

100% BRL medium		150 ml
CM		100 ml
L-Glutamin 200 mM	Gibco/invitrogen 25030-024	1.5 ml
β -Mercaptoethanol (1000x)		0.25 ml
Add LIF (100x)		2.5 ml

DM (depleted medium)

1 x GMEM		100 ml
FCS		10 ml
β -Mercaptoethanol (1000x)		0.1 ml

Water

500 ml bottles Gibco/invitrogen 15230-089

Gelatin (10x)

1 % Gelatin (Sigma G1890) in water, dissolve 5 gram in 500 ml heat in microwave but do not boil, filtrate through 0.22 μ m while still warm.

PBS

500 ml Gibco/invitrogen 14190-169

β -Mercaptoethanol (1000x) *

0.1 M : 0.1 ml 2-mercaptoethanol, 14.2 M (Merck 15433) + 14.1 ml water
Working conc.: 0.1 mM
Note: do not use stock solution longer than one month

Trypsin-EDTA (1x)

100 ml bottle Gibco/invitrogen 45300-019

TVP (1x) *

PBS		477.5 ml
EDTA 40 mM		12.5 ml
Chicken serum	Gibco/invitrogen 16110-033	5 ml
Trypsin 2.5%	Gibco/invitrogen 25090-028	5 ml

TVP (2x)

9.9 ml TVP (1x) plus 0.1 ml Trypsin 2.5%

TVP (10x)

9.1 ml TVP (1x) plus 0.9 ml Trypsin 2.5%

Pen/strep (100x)

Penicillin/Streptomycin (100x) Gibco/invitrogen 15140-114

LIF (100x)

10^5 Units/ml complete medium of ESGRO-LIF (Gibco 3275SB)
= 1 ml in 99 ml CM
Working conc.: 10^3 U/ml

G418 (100x) *

20 mg G418 (Gibco/invitrogen 11811-031) per ml 60% medium, store at 4 °C
Working conc.: 200 μ g/ml

Hygromycin B (100x) *

15 mg Hygromycin B (Calbiochem. 400050 or 400051) per ml 60% medium,
4 °C
Working conc.: 150 μ g/ml

Histidinol (100x) *

250 mM : 53,525 mg L-Histidinol.2HCl (Sigma H6647) per ml 60% medium,

4 °C
Working conc.: 1.0 - 2.5 mM

HAT (100x)

Hypoxanthine 10 mM in H₂O, neutralized with 10 M NaOH, -20 °C
Aminopterin 80 µM in H₂O, -20 °C
Thymidine 2 mM in H₂O, -20 °C

HAT (50x)

HAT Supplement (50x) Gibco 043-01060H 100 ml

Gancyclovir (100x) *

0.1275 mg Gancyclovir (Roche 95B15) per ml 60% medium, 4 °C
Working conc.: 5 µM

Puromycin (1000x)

18 mg Puromycin (Sigma P7255) per 10 ml PBS, -20 °C
Working conc.: 1.8 µg/ml

6-Thioguanine (25x)

2.5 mg 6-TG (Sigma A4660) per 10 ml 60% medium, 4 °C
Working conc.: 10 µg/ml

NOTES

- * Solutions are sterilized by filtration through 0.22 µm Millex-GV
- Only disposables are used, no glassware.
- The quality of water is essential; we use water from Gibco/invitrogen.

2. Antibiotics

			users conc.:
Gentamycine	Gibco/invitrogen	15710-031	20 µg/ml
Kanamycine	Gibco/invitrogen	15160-021	100 µg/ml
Ampiciline			100 µg/ml
Fungizone/amphotericinB	Gibco/invitrogen	15295-033	0.25-2.5 µg/ml
Pen/strep	Gibco/invitrogen	15075-013	100 U/ml