

Medium and reagents for JM8.N4 (C57/Bl6/N) ES cells & feeders

Tissue Culture Protocol

05/01/2009

M10G + LIF medium

- 500 ml GIBCO KNOCKOUT DMEM (10829)
- 50 ml FBS (GIBCO)
- 5 ml 100X GIBCO L-Glutamine
- 5 ml 100X beta-mercaptoethanol (360 microliters/500 ml PBS; filtered, stored at -20°C)
- Murine LIF (ESGRO, Millipore ESG1107, dilute as directed)

Freezing medium:

- Add dimethylsulphoxide (DMSO) (Sigma # D2650) to ES cell medium to a final concentration of 10% (v/v)
- Filter sterilise
Make fresh before use

Trypsin; 2x+G

- To **500ml PBS** (Gibco cat # 14190-144) add **0.1g EDTA** (Sigma cat # 02650) and **0.5g D-glucose** (Sigma cat # G7528 1 kilo)
- Filter sterilize (0.22 µm)
- Add **5ml Chicken Serum** (Gibco cat # 16110-082)
- Add **20ml 2.5% Trypsin** (Gibco cat # 151090-046)
- Aliquot 20ml into universals

Geneticin (G418; Invitrogen Cat No. 11811-031)

C57/Bl6/N JM8.F6 cells are more sensitive to G418 than 129 ES cells: 100 micrograms (active)/ml Geneticin is recommended for trapping experiments and 150 micrograms (active)/ml for targeting experiments.

The concentration of each Lot of geneticin should be titrated to determine the minimum concentration that will kill non-transfected ES cells in 5 days. While ordering Geneticin, request several bottles from the same Lot.

- Dissolve the powder in the appropriate amount of PBS to make a 100mg/ml stock solution (active concentration).
- For example; 5g of powdered geneticin with an active concentration of 740 µg/ml would require a final volume of 37ml of PBS

$$\frac{5000 \text{ mg}}{100 \text{ µg/ml}} \times \frac{740 \text{ µg}}{1000 \text{ µg}} = 37 \text{ ml of PBS}$$

- Add approximately half the final volume of PBS to the Geneticin; swirl bottle until powder is fully dissolved
- Aspirate the solution and transfer to a 50ml tube
- Add approximately quarter of the final volume of PBS to the Geneticin bottle to ensure residue is collected.
- Aspirate the solution and add to the geneticin solution. Mix well. Add PBS to final volume (i.e. 37 ml)
- Filter sterilize (0.22 µm filter)
- Aliquot and store at -20 °C
- Store at 20°C
- Note: When ordering, request several bottles of Geneticin from the same lot

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0.1% Gelatin

- Add 25ml of 2% Gelatin solution (Sigma #g1393) to 500ml PBS (Gibco cat # 14190-144)
Gelatin solution needs to be warmed in the water bath prior to mix.

100x β -mercaptoethanol stock solution (BME):

- 360 μ l BME (Sigma cat. #: M7522) + 500ml PBS (Gibco cat # 14190-144)
- Filter sterilize (0.22 μ m)
- Aliquot and store at -20°C