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Thawing ES Cells

Embryonic Stem Cell Medium (ES)

Dulbecco's modified Eagle's Medium (DMEM) with high glucose (Gibco #11960-044)
To the 500ml bottle add:

- 6mls of GlutaMAX, (GlutaMAX-1, Gibco 35050-061)
- 6ml of diluted ß-mercaptoethanol, 100µM final**

****Note**: Diluted 2-mercaptoethanol is made by adding 70ul of 2-mercaptoethanol (Sigma, M7522) to 100ml of sterile PBS or water.

- 6mls of Sodium Pyruvate, 1mM final (Gibco, 11360-070)
- 6mls of Non-essential amino acids, 100 µM final (Gibco, 11140-050)
- 1000U/ml of LIF (Leukaemia inhibitory factor, Chemicon ESG1107)
- penicillin/streptomycin (Gibco #15140-148, final concentration 50ug/ml each)

Plus the required amount of FBS for the different media types
-normal ES media has 15% which = 95ml of FBS added

Thawing vials of ES cells

- Thaw the vial quickly in warm water
- Transfer the contents of the vial to a tube with at least 5mls of media in it
- Spin at 1000 RMP for 5 min
- Aspirate off the media
- Resuspend the pellet in 4ml of media
- Transfer contents to a 6cm tissue culture treated dish which has feeder cells on it
- Change the media the next day
- ES cells should be passaged every third day with a 1:5 split
- One 6cm dish can be passaged onto two 10cm dishes