XEN (Extra-embryonic endoderm) STEM CELL PROTOCOLS

The protocols and reagents described here are for the maintenance and derivation of extra-embryonic endoderm (XEN) cell lines.

Original reference with some modifications:

> Kunath T, Arnaud D, Uy GD, Okamoto I, Chureau C, Yamanaka Y, Heard E, Gardner RL, Avner P, Rossant J. Imprinted X-inactivation in extra-embryonic endoderm cell lines from mouse blastocysts. Development (2005) 132 (7), 1649-1661.

Media Composition

Base XEN medium

> 500 ml RPMI 1640 (without Glutamine) (Sigma)
> 90 ml fetal bovine serum (final concentration - 15%) (Life Technologies)
> 6.0 ml 100mM sodium pyruvate (final concentration - 1 mM) (Life Technologies)
> 6.0 ml 10mM B-mercaptoethanol** (final concentration - 100 μM) (Sigma)
> 6.0 ml 200mM L-glutamine (final concentration- 2 mM) (Life Technologies)
> antibiotics (pen/strep @ 50 μg/ml each final concentration) if desired

** Stock solution is prepared by adding 70μl β-mercaptoethanol into 100mL of PBS and aliquot at 6.5mL. Aliquots can be stored at -20^{o}C until used.

Thawing XEN cells

Hand-thaw the vial until the suspension is approximately half ice crystals and add contents to 4.0 ml XEN medium and spin to pellet. Re-suspend the cells in 5 ml complete XEN medium. Plate the pellet to 1X 6cm plate. Change medium the next day to get rid of non-adherent cells and continue to feed every two days until cells are ready for passage.

Culturing XEN cells

Culture Conditions: Culture XEN cells are cultured in a standard tissue culture incubator (37^{o}C, 5% CO_2 incubator. Cells are passaged (1:20 to 1:40) every 2-3 days, or when the culture has reached approximately 80% confluency. The medium is normally changed every two days, however, when they reach >60% confluence, the media should be changed daily.

Passaging Cells; When cells reach 90% confluency cells are passaged by trypsin into a single cell suspension. Trypsin with 0.25% trypsin for 3-4 minutes at 37^{o}C with some pipetting up and down is usually sufficient.

Matrix; XEN cells grow well on standard tissue culture dishes if the XEN medium. The dishes do NOT need to be gelatin-coated.

Freezing XEN cells

Prepare 2x freezing medium (50% FBS, 30% XEN medium, 20% DMSO), and cool to 4^{o}C. Harvest and pellet XEN cells as described above. Re-suspend in XEN medium and add an equal volume of 2x freezing medium. Freeze the cells slowly at -70^{o}C overnight and then transfer to liquid nitrogen after 24 to 48 hours. A confluent 100 mm dish has enough cells for about 12 vials.

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