DERIVATION of EXTRA-EMBRYONIC ENDODERM (XEN) STEM CELL

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.

Collection of embryos

XEN cell lines described here are derived from 3.5dpc mouse blastocysts. Mice of interest are mated either through natural mating or super-ovulated. At the required time point (E3.5) the uterus is flushed and 3.5dpc blastocysts are collected and transferred to KSOM until plating.

XEN derivations

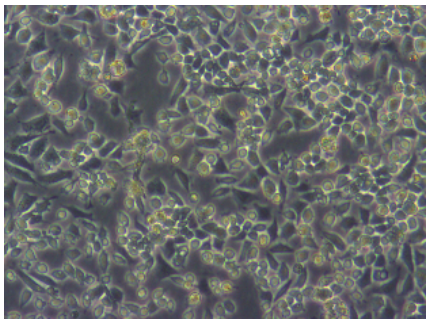
Plating blastocysts: Using sterile conditions, place one blastocyst per well of a 4-well plates containing XEN cell media as described in the XEN stem cell protocol and culture cells at 37^{o}C, 5% CO_2.

Time line of hatching:

- The blastocysts should hatch and attach to the wells in 24 to 36 hours (day 2).
- On day 3, a small outgrowth is formed from each embryo. Feed each culture with 500μl fresh medium.
- Continue to feed outgrowth with XEN media every 2-3 days.
- Around day 6 XEN cells should start to appear surrounded the undisturbed outgrowth.
- Continue to allow XEN cells to populate from the blastocyst until approximately 50% of the well contains XEN cells.

Disaggregation of hatching: Once 50% confluency is achieved embryos are ready to be passed. Remove the medium and wash the cells with PBS. Gently remove the attached embryo from the well using a P10 pipette. Aspirate the remaining PBS, add 0.1% trypsin/EDTA (100μl), and incubate for 5 minutes at 37^{o}C, 5% CO_2. Using a P200 dissociate the cells into a single cell suspension. Immediately stop the trypsinization by adding 100μl XEN media transfer the entire volume to a new 6 cm plate in XEN media. Each well should be treated clonally and passed into an individual new well. Return cells to incubator and change medium every 2 days until XEN cells reach 90% confluency.

Passing XEN cells: Between days 4-5 XEN cells are ready to passage. To passage cells aspirate the medium and wash XEN cells with PBS. Aspirate the PBS, add trypsin/EDTA (500μl), and incubate for 5 minutes at 37^{o}C/5% CO_2. Stop trypsinization by adding XEN and pipetting up and down to get a single-cell suspension. Transfer cells to 1X10 cm plate.



Maintenance: Feed the cells every two days with XEN media. Follow the XEN Stem Cell protocol provided on our website to expand cells for freezing. After one or two more passages cells can be frozen and stored.

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