

## NBP1-41162 Protocol

### Protocol specific for v6.5 Mouse embryonic stem cells (NBP1-41162)

[[URL: [https://www.novusbio.com/products/v65-mouse-embryonic-stem-cells\\_nbp1-41162](https://www.novusbio.com/products/v65-mouse-embryonic-stem-cells_nbp1-41162)]] [[Caption:v6.5 Mouse embryonic stem cells]]

Protocol Specific for v6.5 Mouse embryonic stem cells

#### Growing v6.5 mouse ES cells

This protocol is written for growing cells in T25 tissue culture flasks, please make changes accordingly for flasks of different sizes.

ES cells are routinely cultured in ES medium in the presence of LIF on a mitotically inactivated MEF feeder layer grown on gelatin.

#### 1. Media:

ESL1000 for ES cells:

DMEM-Hi glucose 425 ml (Caisson Labs, DML10-500ML)

FBS 75 ml (biowest, US1520)

100 X non-essential amino acid 5 ml (Millipore EmbryoMax(R) TMS-001-C)

200 mM L-Glutamine 5 ml - (Sigma G7513)

100% beta-mercaptoethanol (100X for ES cells) 5 ml (Millipore EmbryoMax(R) ES-007-E)

10 ng/ml LIF (R&D Systems 8878-LF)

\*\* or use StemXVivo Mouse Pluripotent Stem Cell Media Kit instead (R&D Systems, cat# CCM025)

MEF Media for embryonic fibroblasts:

DMEM-Hi glucose 450 ml (Caisson Labs, DML10-500ML)

FBS 50 ml (biowest, US1520)

100 X non-essential amino acid 5 ml (Millipore EmbryoMax(R) TMS-001-C)

200 mM L-Glutamine 5 ml - (Sigma G7513)

100% beta-mercaptoethanol (100X for ES cells) 5 ml (Millipore EmbryoMax(R) ES-007-E)

#### 2. Preparation of gelatin coated tissue culture flasks:

To make gelatinized flasks, distribute a thin layer (about 2ml per T25 flask) of distilled water with 0.1% gelatin (Sigma) onto a T25 tissue culture flask and incubate at 37 degrees Celsius for 1 hour. Remove the gelatin solution and begin plating the stem cells.

#### 3. MEF feeder flasks:

According to the Culture of Animal Cells textbook by R. Ian Freshney, MEF cells used as a feeder layer should not exceed 6 passages prior to treating with Mitomycin C.

Maintain MEF cells in MEF media for embryonic fibroblasts. The thawed MEF cells can be grown and maintained in a regular T25 tissue culture flask and when confluent, transferred to a T150 flask. Gelatin is not needed for the culture MEF feeder cells.

##### a. Mitotic inactivation (Mitomycin C treatment) for preparation of ES feeder layers:

At confluence, Mitomycin C is used as a treatment to halt cell division. Use the procedure below to prepare fresh MEF feeder layers.

\*Plate mitomycin C treated MEFs in a gelatinized T25 at least one day but not more than 1 week before plating ES cells on the feeder.

3.1 To one T150 tissue culture flask of confluent MEF cells: remove regular growth medium and add 40 ml of fresh MEF medium containing 40ul of Mitomycin C (Sigma, catalog# M4287-2MG) and incubate overnight.

3.2 Remove mitomycin C containing medium and wash twice with PBS, trypsinize, resuspend and replat by dispensing 2ml of MEF cell split into desired number of T25 gelatinized flasks. Note that for this step, a split ratio of about 1:1 or a bit less should be used. The reasoning behind the 1:1 split ratio is to achieve the best feeder cell density. The cells should almost completely cover the bottom of the flask but with enough space left for the ES cell colonies to spread out a bit. As it directly affects the growth of the ES cells, feeder layer quality is extremely important.

#### 4. Thawing ES cells from -80 C or Liquid N2:

Thaw a tube of  $2 \times 10^6$  ES cells in 37 C water bath for 1-2 minutes. During this time, prepare a 15-ml tube, add 10ml warm ESL1000 media. Mix the thawed cells with warm media in the 15-ml tube by adding the thawed cells to the side of the conical tube of warm media just above the buffer level and rotate the conical tube slowly and pipette slowly so the cells fall into the media gradually. Spin down cells at 1000 rpm for 5 minutes. Aspirate off the media carefully without touching cell pellets, add 8 ml fresh ESL1000 media and gently re-suspend the pellet, plate onto a T25 tissue culture flask with MEF feeder cells grown on gelatin.

#### 5. Passaging cells:

After cells settle down (in 3-5 days), remove the media and replace with fresh ESL 1000 media. At 70-80% confluency, aspirate off the media, wash once with Hank's buffered saline or PBS with 1 mM EDTA, add 2ml TrypLE (Gibco) to a T25 flask, incubate at 37 C for 1-2 minutes. Add 2 ml ESL1000 media to the flask, pipette to dislodge cells and transfer to a sterile tube. Gently pellet the cells and resuspend in media, transfer approximately  $0.25 \times 10^6$  cells per well of a 6 well plate containing gelatin. Medium is changed every day and cells are usually split at a 1 to 4 or 5 ratio in 2 days.

#### 6. Freezing cells:

Freeze cells in 1 part of fresh media and 1 part of 2 X freezing media (60% DMEM, 20% FBS, 20% DMSO). Use cryo safe tube. Save tubes in a Styrofoam box at -80 C. For long term storage, move them to liquid nitrogen a few days later.