BTBR $T^+ Itpr3^{tf}/J$-PB60.6 mES cells

**Description**

The BTBR $T^+ Itpr3^{tf}/J$-PB60.6 mES cells were provided by Ted Choi, PhD of Predictive Biology Inc. The BTBR $T^+ Itpr3^{tf}/J$ mouse is a specialized strain with a black and tan coat that has a complete absence of the corpus callosum, low hippocampal commissure, and exhibit several symptoms of autism. For further strain information, please see the JAX® Mice Database at www.jax.org/jaxmice/strain/002282. The BTBR $T^+ Itpr3^{tf}/J$-PB60.6 mES cells are being provided at passage 9.

mES cells are cultured in mES cell medium as described below and replenished daily. mES cells are maintained on irradiated MEFs from C57BL/6J or B6D2F1/J mice. Therefore, each vial of mES cells contain a small number of irradiated fibroblast feeder cells.

**Quality Assurance**

JAX® mES cells are tested for bacterial and fungal growth and for the presence of mycoplasma using a PCR detection system. Cell culture contaminants were not detected. The mouse strain of the cell line was confirmed by SNP analysis. mES cells will spontaneously differentiate in the absence of mouse embryonic fibroblasts (MEFs) and supplemental growth factors, which together act as inhibitors of differentiation. Germ line testing of BTBR $T^+ Itpr3^{tf}/J$-PB60.6 mES cells is currently underway. JAX makes no guarantee that this cell line can be used to generate targeted clones or, if targeted clones are created, that they will be germ line competent.
**Cryopreservation Medium**
90% FBS and 10% DMSO, prepare in quantities that can be used in a 2-week period.

**Thaw and culture of mES cells**

1. Plate MEF feeder cells one day in advance of thawing and plating the mES cells. When plating MEFs at a concentration of 5 x 10^6 cells/vial, they will cover 1 x T75 tissue culture flask, 3 x T25 tissue culture flasks or 2 x 6-well plates.
2. Thaw the vial of mES cells rapidly in a 37° C water bath.
3. Transfer contents to a 15 ml sterile conical tube.
4. Slowly add 5 ml mES cell medium with mixing.
5. Centrifuge tube at 1200 rpm for 5 minutes in a table top centrifuge.
6. Aspirate and discard supernatant. Vortex pellet and suspend in 2 ml mES cell medium, count mES cells and add to a MEF monolayer (0.5-1 x 10^6 mES cells per 1 x 10^6 MEFs). Allow mES cells to attach overnight in 95% air; 5% CO2 at 37° C.
7. Next morning, remove medium and replace with fresh mES culture medium. Change medium daily.

**Subculturing mES cells at a ratio of 1:3 to 1:5**

1. Remove and discard the mES cell culture medium.
2. Rinse the adherent mES and MEF cells with small amount of 0.05% Trypsin/0.02% EDTA to remove traces of medium and serum.
3. Add a quantity of 0.05% Trypsin/0.02% EDTA to cover the cell layer, usually equivalent to the amount used to culture the cells.
4. Incubate cells with 0.05% Trypsin/0.02% EDTA for 5-7 minutes, watching for cell lift using the inverted microscope.
5. Collect the cells in 0.05% Trypsin/0.02% EDTA and then rinse the culture dish with an identical amount of mES cell medium. Collect both aliquots (trypsinized cells and rinse) in one tube.
6. Centrifuge tube contents, suspend in either freezing medium for cryopreservation or in culture medium and transfer to a new culture vessel that contains a monolayer of feeder cells. Incubate in 95% air; 5% CO2 at 37° C, change medium daily.