B6-White™ Murine ES Cell Line

Description:
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Product Overview:
The generation of gene-modified mice, created by homologous recombination in embryonic stem (ES) cells, has become a fundamental tool for analyzing gene function. The influence of genetic background on phenotype has been shown to be an important consideration in selection of a mouse model. Furthermore, the time required to achieve congenic status, which can be 18-24 months has further stimulated demand for targeting in pure inbred strains including C57BL/6 (B6) mice.

Millipore's B6-White Murine ES cell line is the first commercially available C57BL/6 tyr(c-2J) albino line that allows for rapid coat-color determination of successful chimerism in the C57BL/6 mouse strain. When B6-White ES cells are injected into C57BL/6 blastocysts, chimeric mice are easily identified by their coat color (a mix of black and white patches), whilst non-chimeric littermates are black. These cells allow for the efficient generation of gene-targeted mice in a pure C57BL/6 genetic background, thus providing more experimental flexibility. Additionally the use of these cells can lower production costs by eliminating the need to maintain an albino blast donor colony, in order to assess chimerism by coat color when targeting in the C57BL/6 strain.

For germline transmission confirmation by coat color, it is recommended that male chimeras are mated with homozygous females of the B6-white strain (strain name: C57BL/6J-Tyr(c-2J/J - available from Jackson Labs, stock number 000058).

STRAIN:C57BL/6-tyr(c-2J) albino mice

GENDER: Male

KARYOTYPE: 40XY

Key Applications:
Stem Cell Culture

Application Notes:
PluriStem B6-White ES cells are fast growing and should be maintained as small dense, but not confluent cultures. The colonies will be tightly packed with phase bright borders. Optimal results are achieved when the cells are maintained as small colonies at high density, fed daily and passaged 1:3 to 1:5 every other day.

It is recommended that B6-White ES cells be cultured on a monolayer of mitotically inactivated primary mouse embryonic fibroblast cells (MEFs) in the presence of 1000U/mL ESGRO® mLIF Medium Supplement (ESG1106, ESG1107).

Culture Notes:
- Incubator settings: 7.5% CO₂ in humidified air, 37°C
- Replace the media daily (please refer to the recommended media formulation below),
- Passage every other day
- Trypsinize gently using 0.05% Trypsin/EDTA

Microinjection Notes:
Inject 8-12 cells/C57BL/6 blastocyst.
Day 0
Prepare the following culture dishes containing a monolayer of mitotically inactivated MEF feeder cells:

- 1 x 25cm² flask
- 1 x 75 cm² flask
- 3 x 10 cm dishes

Day 1
Thaw one vial of B6-White ES cells directly into a 25cm² flask containing a confluent layer of inactivated MEF cells and 5 mls of freshly prepared B6-White ES cell media.

- Replace the MEF cell medium with 5.5 mls of B6-White medium (see below), and allow it to equilibrate in a 37°C incubator 1 hour before thawing the ES cells.
- Thaw one vial of B6-White ES cells by gently shaking the tube in a 37°C water bath. When the contents of the tube have thawed, spray the vial with ethanol, dry the outside of vial, and aseptically transfer the contents of the vial to the flask.
- Place the flask in a 37°C incubator overnight.

Day 2
Passage the B6-White ES cells to a 75 cm² flask, containing a confluent layer of inactivated MEFs and 15 mls of pre-warmed B6-White ES cell medium.

- Examine the ES cells under the microscope. Many small phase bright ES cell colonies should be visible.
- Replace the MEF cell medium in the prepared 75 cm² flask with 15 mls of B6-White ES cell medium, and allow the medium to equilibrate in a 37°C incubator for 1 hour before passaging the ES cells.
- Aspirate the medium from the 25 cm² flask containing the B6-White ES cells and rinse with 3 mls of PBS.
- Aspirate the PBS and add 1.5 mls of 0.5% Trypsin/EDTA, place the flask in an incubator for 5 minutes or until the ES cells are dissociated.
- Add 5 mls of B6-White ES medium and gently titurate the contents of the flask.
- Transfer the cell suspension to the prepared 75 cm² flask.
- Place the flask in the 37°C incubator overnight.

Day 3
Electroporation.

- Gently trypsinze the B6-White ES cells, as described above, and follow your preferred electroporation protocol.
- Plate the electroporated cells on the prepared 10 cm dishes and begin selection 24 hours following electroporation.

B6 WHITE ES CELL MEDIUM PREPARATION (250 mL final volume):
Mix all ingredients in the top of a 250 mL filter (0.22µm PES unit, Millipore Cat. No. SCGPU02RE) and filter sterilize. Store at 4°C. Discard unused media 7-10 days after preparation.

- IMDM - 190 mL (Millipore cat. no. SLM-063-B)
- FCS-ES Cell qualified - 50 mL (Millipore cat. no. ES-009-B or -C)
- L. glutamine 200mM - 2.5 mL (Millipore cat. no. TMS-002-C)
- Non-Essential Amino Acids - 2.5 mL (Millipore cat. no. TMS-001-C)
- Pen/Strep - 2.5 mL (Millipore cat. no. TMS-AB2-C)
- Na Pyruvate 100mM - 2.5 mL (Millipore cat. no. TMS-005-C)
- 2-ME (100X), 0.2mM final conc. - 5.0 mL (Millipore cat. no. ES-007-E)
- ESGRO mLIF Medium Supplement - 25uL of ESG1107 or 250uL of ESG1106 (Millipore cat. no. ESG1107 or ESG1106)

Appearance Notes:
With this line the pup fur seems to be more pink than white. When the pups are born one can see that the pups have white blotches on their skin, but as they mature the black and white fur seem to mingle and one ends up with a “smoky” pattern with blotches of white. We suggest that one breed all the chimeras that one obtains because germline transmittance by these low coat color chimeras is common.
Usage Statement:
Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

Cell Line Type:
Embryonic Stem Cells

Presentation:
Cells are supplied frozen in basal medium supplemented with 10% FCS and 10% DMSO Storage Conditions: Place vials in the vapor phase of liquid nitrogen storage immediately upon receipt until it is convenient to proceed to subculture.

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