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B6-WHITE™ MURINE ES CELL LINE

CATALOG NUMBER:	SCR011	**QUANTITY:**	5 x 10^6 cells, passage 10
LOT NUMBER:		**CONCENTRATION:**	2.5 x 10^6 cells / vial

BACKGROUND:

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.

DESCRIPTION:

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), while 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

KARYOTYPE:

40,XY [20]
Apparently NORMAL Male Mouse Karyotype

Banding Technique: GTL
Band Resolution: Good
Metaphases Counted: 20
Analyzed: 6
Karyotyped: 6
Interpretation: Cytogenetic analysis was performed on twenty G-banded metaphase cells from mouse cell line SCR011 p11 and all twenty cells demonstrated an apparently normal male karyotype. No abnormal cells with trisomy 8 and/or trisomy 11 were detected.

PATHOGEN TESTING:

Cells tested negative for mycoplasma and other pathogens by infectious disease PCR.

PRESENTATION:

Cells are supplied frozen in basal medium supplemented with 10% FCS and 10% DMSO

STORAGE/HANDLING:

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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B6-WHITE ES CELL MEDIUM: Mix all ingredients in the top of a 250 mL filter (0.22 µm PES unit, Cat. No. SCGPUO2RE), and filter sterilize. Store at 4°C. Discard unused media 7-10 days after preparation.

IMPORTANT NOTE: For successful B6-White ES cell culture, the following medium formulation is highly recommended.

Component	250 mL Final Volume	Millipore Cat. No.
IMDM, with 25mM HEPES, 3,024mg/L $NaHCO_3$ & L-Glutamine, w/out Alpha-thioglycerol & beta-mercaptoethanol	185 mL	SLM-063-B
FCS-***ES cell qualified***	50 mL	ES-009-B, ES-010-B, ES-011-B
L. glutamine (100x), 200mM	2.5 mL	TMS-002-C
Non-Essential Amino Acids (100x)	2.5 mL	TMS-001-C
Pen/Strep	2.5 mL	TMS-AB2-C
Na Pyruvate (100x), 100mM	2.5 mL	TMS-005-C
2-mecaptoethanol (100x), 0.2mM final conc.	5.0 mL	ES-007-E
ESGRO® mLIF Medium Supplement (1000 U/mL final conc.)	25 µL ESG1107 or 250 µL ESG1106	ESG1107 (1 x 10^7 units) or ESG1106 (1 x 10^6 units)

SUBCULTURING: 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 are cultured on a monolayer of mitotically inactivated primary mouse embryonic fibroblast (PMEF) feeder cells in the presence of 1000U/mL ESGRO mLIF Medium Supplement (ESG1106, ESG1107).

Culture Notes:

- Incubator settings: 7.5% CO_2 in humidified air, 37°C
- Replace the media daily (please refer to the recommended media formulation above).
- 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 PMEF feeder cells:

- 1 x 25 cm^2 flask
- 1 x 75 cm^2 flask
- 3 x 10 cm dishes

Day 1

Thaw one vial of B6-White ES cells directly into a 25 cm^2 flask containing a confluent layer of inactivated PMEF cells and 5 mL of freshly prepared B6-White ES cell medium.

- Replace the PMEF cell medium with 5.5 mL of B6-White ES cell medium (see above), and allow it to equilibrate in a 37℃ incubator 1 h our before thawing the ES cells.
- Thaw one vial of B6-White ES cells by gently shaking the tube in a 37℃ 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℃ incubator overnight .

Day 2

Passage the B6-White ES cells to a 75 cm^2 flask, containing a confluent layer of inactivated PMEF cells and 15 mL 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 PMEF cell medium in the prepared 75 cm^2 flask with 15 mL of B6-White ES cell medium, and allow the medium to equilibrate in a 37℃ incubator for 1 hour before passaging the ES cells.
- Aspirate the medium from the 25 cm^2 flask containing the B6-White ES cells and rinse with 3 mL of PBS.
- Aspirate the PBS and add 1.5 mL of 0.5% Trypsin/EDTA, place the flask in an incubator for 5 minutes or until the ES cells are dissociated.
- Add 5 mL of B6-White ES cell medium and gently titurate the contents of the flask.
- Transfer the cell suspension to the prepared 75 cm^2 flask.
- Place the flask in the 37℃ 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.

APPEARANCE NOTES:

With this line the pup fur may 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 all chimeras obtained are bred because germline transmittance by low coat color chimeras is common.

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REPRESENTATIVE DATA: Germline transmission data generated using wild type B6-White ES cells:

Wild type	Blastocysts injected	Live pups (%)	Chimeric pups (%)	Male chimeras (%)	Germline
A	40	14 (35%)	3 (5%)	2 (66%)	1 (50%)
B	78	40 (51%)	3 (7%)	3 (100%)	2 (70%)
C	76	32 (42%)	6 (20%)	5 (83%)	2 (40%)

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