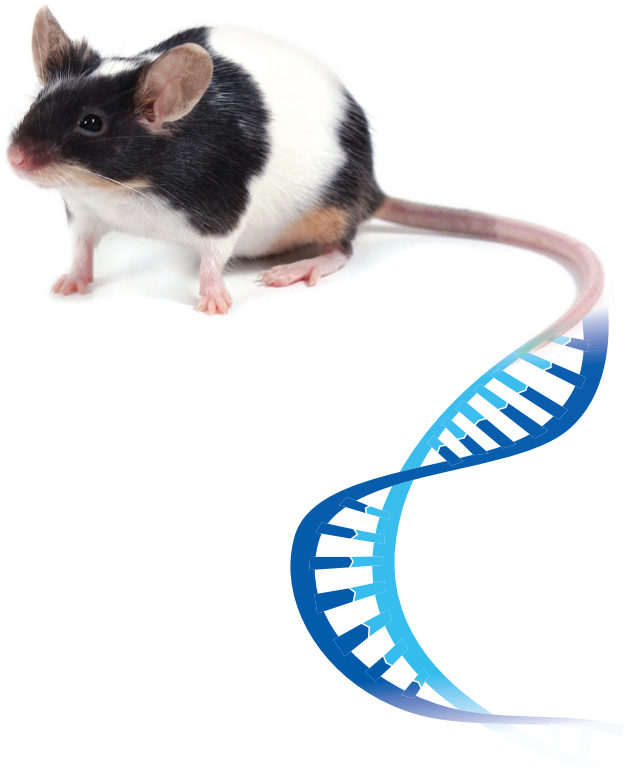
The Genetically Modified Mouse: An Indispensable Tool for Disease Modeling

Five little-known facts about laboratory mouse models

As transgenic mouse core laboratories know well, mouse models are a powerful means of determining the function of mammalian genes in the context of whole organisms—especially since over 95% of the mouse genome is similar to the human genome. However, you may not know that:

1. Laboratory mouse strains are descended from ancestors in modern-day Pakistan[1].
2. The first inbred strain of lab mouse was created in 1909. It was named DBA for its coat color: dilute brown non-agouti[1].
3. There is a mutant mouse actually named"Bad Hair Day" (Bhrd)[2]. Someone get this little guy a stylist!
4. Male mice, when treated with anticancer drugs, have been shown to pass on DNA instability to their offspring[3].
5. The founder of Jackson Laboratories, C. C. Little, once had lunch with Walt Disney to request that an animated film be created linking Mickey Mouse and the laboratory mouse. The request was denied[4].



So, what's new?

Mouse geneticists have come a long way in the past century. Some of the newest, most exciting genetically modified mouse models include:

April 2014	Compared to previous mouse models of Alzheimer's disease, new mouse models with knockin mutations of amyloid precursor protein more accurately mimic amyloid beta accumulation and memory loss seen in human patients[5].
July 2014	Mouse model of dystonia, a progressive neurodegenerative disorder. In the mouse model, a conditional deletion in the TOR1A gene resulted in accurate recapitulation of the abnormal twisting movements characteristic of the human disorder, accompanied by neurodegeneration in very specific regions of the brain[6].
September 2014	The first CRISPR-Cas9 knockin mouse was used to model lung adenocarcinoma using *in vivo* genome editing with a single delivery of a adeno-associated virus[7].

In this research focus, we will summarize the approaches used to generate genetically modified mice and outline some important considerations for successfully performing key steps.

EMD Millipore Corp. is a subsidiary of Merck KGaA, Darmstadt, Germany

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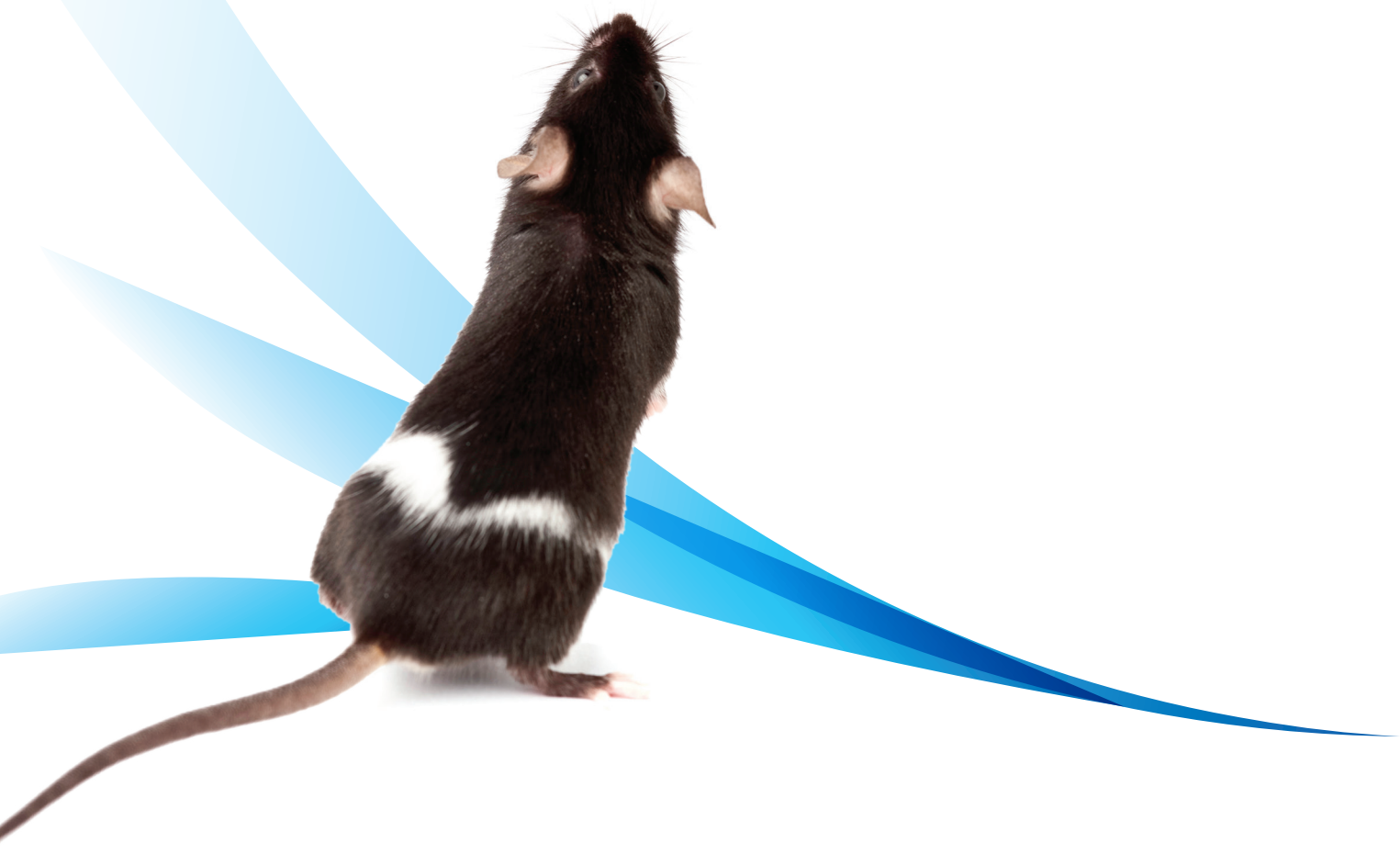


General Procedure

Let us look at the method for generating mouse models using genetic manipulation of ES cells. It is outlined below:

ES Cell Culture

1. Embryonic stem (ES) cells are cultured from mouse blastocysts.

Genetic Manipulation

2. Construction of a targeting vector (traditional or CRISPR/Cas9 system)

3. Transfection or transduction of targeting vector into ES cells or microinjecting zygote directly (CRISPR/Cas9 only)

4. Homologous recombination

5. Selection of targeted ES cells

Mouse Embryo Handling and Culture

6. Targeted ES cells are injected into the blastocyst

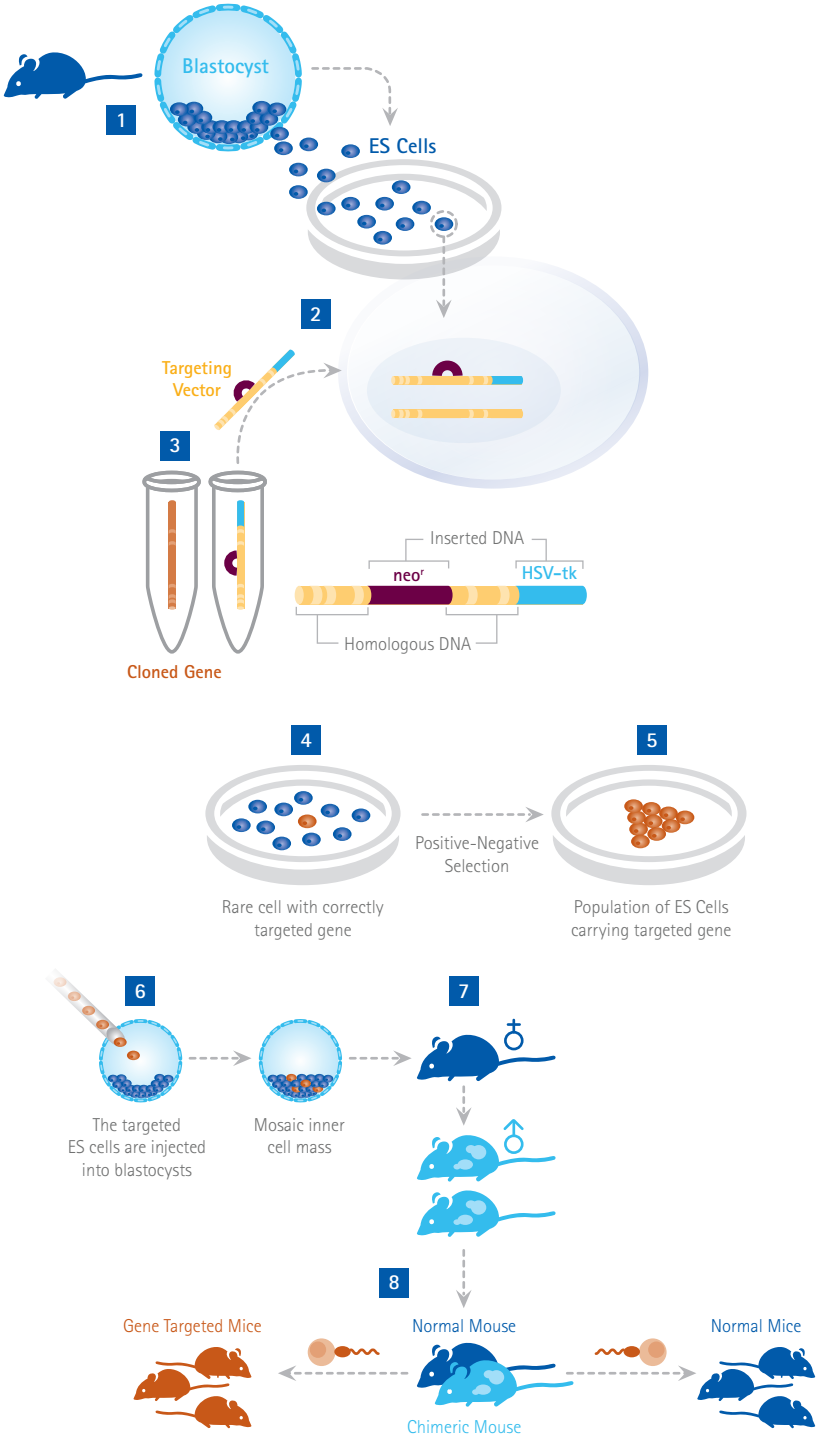
7. Blastocyst is injected into the mouse, which acts as a surrogate mother

Mouse Birth and Breeding

8. Chimeric mice are produced; they are mated with normal mice

9. Gene-targeted mice are born

Genotyping



PRODUCTS

- PluriStem® Murine ES Cells (multiple different strains)
- B6-White™ ES cell line, the first commercially available C57BL/6 tyrc-2J albino line that allows for rapid coat-color determination of successful chimerism in C57BL/6 mice.
- Trypsin
- Accutase® reagent
- Electroporation Buffer
- Transfection reagents
- ESGRO®/LIF supplement
- EmbryoMAX® PMEF feeder cell lines
- RESGRO™ culture medium
- ESGRO Complete™/ESGRO®-2i medium/supplement
- Qualified FBS (ES-009-B)
- DMEM (low bicarb), NEAA, β-mercaptoethanol, L-glutamine, pen-strep, nucleosides, gelatin
- Freezing media
- Selection agents: Puromycin

Hormones

- PMSG
- hCG

Culture Media

- KSOM
- M16
- CZB
- HTF

Holding Media

- M2
- FHM

Special Purpose Media

- Acidic Tyrode's Solution

PCR Reagents

- Including KOD DNA polymerases and OmniPur® PCR Plus agarose

Figure 1.

General procedure for generating genetically modified mice. Pluripotent ES cells can be purchased or isolated from mouse blastocysts. The ES cell genome can be modified in two ways: 1) add expression of an exogenous gene to the ES cell 2) disrupt the expression of an endogenous gene. In both cases, the targeting vector contains sequences homologous to the target gene as well as sequences changing the target and enabling positive/negative selection. Modified ES cells are microinjected into blastocysts. The new CRISPR/Cas9 genome editing system provides the option of directly microinjecting zygotes with the targeting mRNAs along with the Cas9 mRNA. Blastocysts are injected into the mouse to generate chimeric mice. These are mated with normal mice to generate mice with the desired genetic modification.

CRISPR/Cas9 Genome Engineering

While several genome editing tools have been developed in recent years, including zinc finger-based strategies and TALENs (transcription activator-like effector nucleases), none have been as efficient as the CRISPR/Cas9 system, which consists of an RNA-guided DNA endonuclease (Cas9) and corresponding guide RNAs (CRISPRs). Using this system, researchers were able to achieve one-step generation of mutant mice with both alleles of multiple genes being knocked out[8]. Mice that bore conditional alleles and reporter genes were also created[9], and the protocol required as little as two or three weeks. Notably, the procedure does not require the sometimes-arduous process of creating modified ES cells (Figure 2)[10]. With the development of the Cas9 knockin mouse, it is expected that more and more laboratories will be choosing the CRISPR/Cas9 system for generating mouse models.

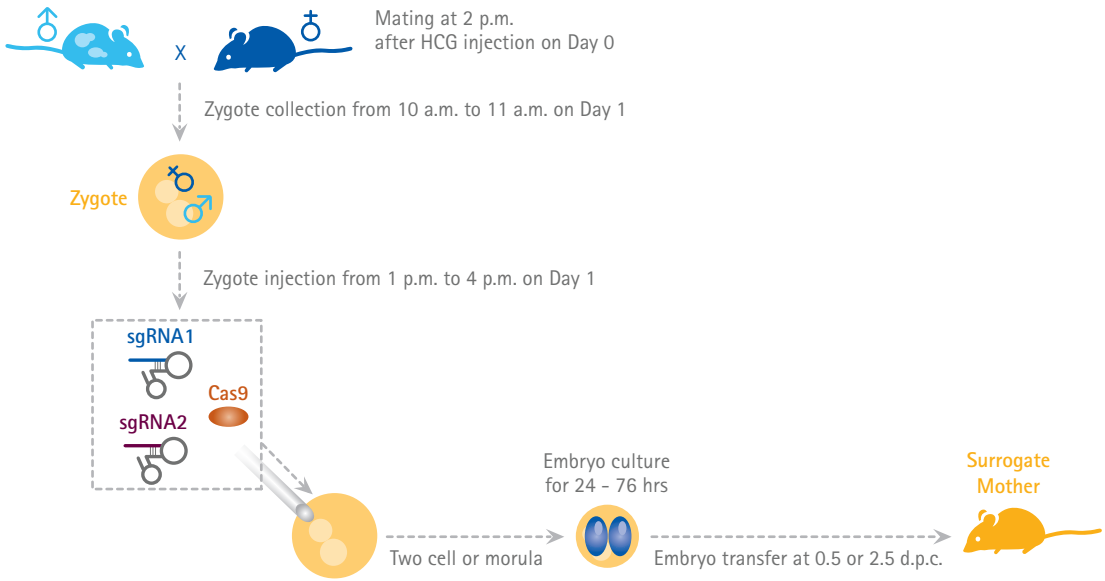


Figure 2.

Using CRISPR/Cas9 genome editing by coinjecting Cas9 mRNA and guide RNAs to knock out multiple gene targets at once. (Adapted from Yang H, Wang H, and Jaenisch R. Nat Protoc. 2014 Aug;9(8):1956-68.)

Tips for a successful mouse model project

1. **Know the purpose of the experiment and do your research.**
   Generating the right mouse requires fully understanding the hypothesis being tested. For example, an investigator might wish to test the hypothesis that mutating a transporter protein in the liver might mitigate the hepatotoxic effects of a particular drug. Knowing this hypothesis will help the team select the appropriate mouse strain and design appropriate targeting vectors and biological controls. Thoroughly read the literature and all mouse genetics informatics databases to determine if an appropriate mouse model already exists, and if so, whether it can be obtained from the creators.

2. **Determine the spatial and temporal environment in which gene expression changes should occur.**
   In light of the hypothesis being tested, determine whether the gene modification should be controlled by a tissue-specific, lineage-specific or inducible promoter. Some transgenes or gene knockouts may be lethal to the embryo; these are cases in which inducible promoters are required.

3. **Choose an appropriate mouse strain.**
   Each strain of inbred mice has distinguishing characteristics and may be uniquely suited to particular areas of research. For example, BALB/c mice are suitable for immunology research, but, due to inherent neurodevelopmental defects, may not be suited for generating models of nervous system disorders. NOD mice are suitable for models of autoimmunity and metabolic syndrome, but, due to their short lifespan, have limited utility for studies of aging.

4. **Use well-characterized promoters and design restriction sites into the construct so that you have the option of removing vector sequences that inhibit transgene expression.**
   If expressing an exogenous gene, ideally, the targeting vector should include a promoter that has previously been used to generate transgenic mice. Also, try to determine if vector sequences might inhibit expression. Always test the construct in cultures of the cell type of interest to confirm expression.

5. **Include a strong polyadenylation sequence (such as SV40 polyadenylation sequence)**
   Include an intron sequence in the construct so that the transcript will be spliced. Because the splicing machinery may mediate transcription-translation coupling, gene expression may be enhanced by inclusion of an intron.

6. **Optimize rate of homologous recombination.**
   Increasing the length of the homologous region in your targeting construct may increase the rate of recombination. The chromatin structure of your target may affect recombination frequency, as well as genetic background.

7. **If transfecting ES cells, use a high-efficiency transfection reagent.**
   Try numerous transfection reagents or methods to determine which generates transfected ES cell clones with the greatest efficiency and lowest toxicity.

8. **Establish a screening assay, such as a PCR assay or Southern blot.**
   Ideally, your screening assay should employ a probe or primers bearing sequences not present in the unmodified cell.

9. **Develop an assay for gene expression.**
   Potential assays include Western blot, ELISA, Luminex® assay, immunohistochemistry, flow cytometric assay, RT-PCR, or a SmartFlare™ live cell RNA assay.

10. **Develop a reliable genotyping strategy.**
    Using tail biopsies or ear punch tissue, develop a rapid protocol for isolating genomic DNA and assessing genotype using PCR. Some DNA polymerases, such as KOD Xtreme™ DNA polymerase, are particularly suited to genotyping, because they can accurately and efficiently amplify DNA from crude samples, such as tail digests. For such polymerases, it may not be necessary to purify the tail digest before adding 1 µL or less of the digest to the PCR reaction. Tail biopsies frequently yield large amounts of DNA; if PCR results in no visible bands, it may be that an overabundance of template sequence compared to primer concentration is inhibiting amplification.

11. **Follow best practices for breeding transgenic mice to maintain stocks.**
    The number and characteristics of breeding pairs required to maintain your supply of modified mice depend on many factors. Some of these factors include strain-specific behavior, effects of the particular mutation or transgene, colony temperature and humidity, light source and light cycle, vibrations, noise, odors, handling, nutrition, barometric pressure, and overall health[11].

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Tools for your transgenic mouse research

Efficient procedures for the *in vitro* culture and maintenance of pluripotent ES cells can be vital to successful mouse model generation. EMD Millipore provides the largest and most comprehensive range of products for your mouse ES cell culture needs. Highlights include unique mLIF formulations such as ESGRO® medium supplement for the maintenance of pluripotent mouse ES cells; ESGRO Complete™ PLUS serum-free medium for the maintenance and derivation of mouse ES cell lines in the absence of FBS and feeder cells; and RESGRO™ culture medium for the rescue of partially differentiated ES cell lines and improved ES cell derivation.

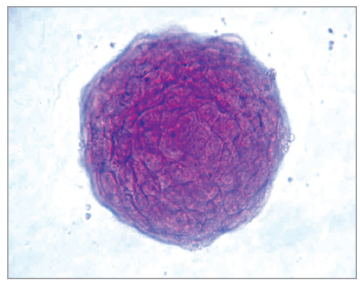
Mouse Embryonic Stem Cell Expansion

ESGRO® mLIF Supplement

Maintaining pluripotency for over a decade

For over a decade, stem cell researchers have trusted their cultures with ESGRO® mLIF supplement for maintaining the pluripotent state of their mouse ES cell lines. The gold standard for undifferentiated mouse ES cell culture, ESGRO® mLIF features:

- Consistent inhibition of ES cell differentiation
- Convenient format, supplied in active units/mL
- No batch-to-batch variation
- Flexibility; supports feeder-free and feeder-based cell culture



Alkaline phosphatase staining shows that ESGRO® supplement inhibits mES cell differentiation.

Used by the Most Influential Stem Cell Researchers...

1. **Yamanaka, S. et al.** Aggregation of embryonic stem cells induces Nanog repression and primitive endoderm differentiation. J Cell Sci. 2004 Nov 117:5681-6.
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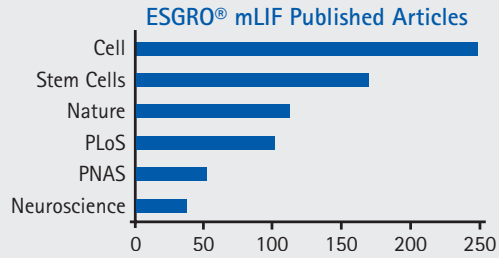
...in the Top Peer-Reviewed Stem Cell Journals



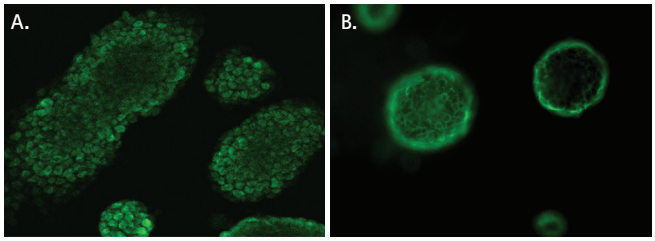
Total Publications using ESGRO® mLIF:

2500 and Counting

Product Description	Units	Cat. No.
ESGRO® Mouse LIF Medium Supplement	10^6	ESG1106
ESGRO® Mouse LIF Medium Supplement	10^7	ESG1107

ESGRO Complete™ PLUS Media and Reagents

The ESGRO Complete™ system is the first to enable serum-free and feeder-free culture of mouse ES cells. The cornerstone of this system is the ESGRO Complete™ clonal grade medium, which supports the self-renewal of mouse ES cells by providing the basic nutrients normally supplied by serum and feeders in the traditional culturing method. These nutrients include hormones, vitamins, the growth factors mLIF and BMP4, as well a selective GSK3β inhibitor for enhanced mouse ES cell growth and viability at clonal densities in serum-free conditions.



To confirm pluripotency of ES cells after culturing in ESGRO Complete™ PLUS medium, cells were immunostained for Oct-4 (A) and SSEA-1 (B) after 3 passages.

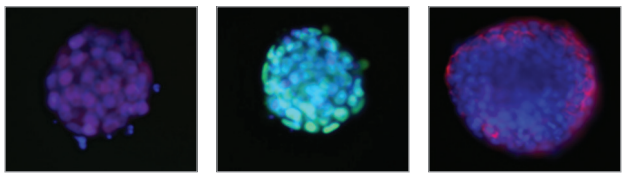
Product Description	Cat. No.
ESGRO Complete™ PLUS Clonal Grade Medium	SF001-500P, SF001-100P
ESGRO Complete™ Basal Medium	SF002-500, SF002-100
ESGRO Complete™ Serum-Free Cell Culture Freezing Medium	SF005
ESGRO Complete™ Accutase	SF006
ESGRO Complete™ Gelatin Solution	SF008
ESGRO Complete™ Trypsin Solution	SF007
ESGRO Complete™ Adapted C57/BL6 Mouse ES Cell Line	SF-CMTI-2

ESGRO®-2i Medium

Mouse ES cells misbehaving?
"Ground" them with ESGRO®-2i medium or supplement!

Based on the principle of using LIF along with GSK3β and Mek 1/2 small molecule inhibitors to establish and sustain ES cells in a "ground state of pluripotency[1-3]", EMD Millipore now offers ESGRO®-2i medium or supplement*, designed to:

- Maintain "naïve state" or "ground state" culture conditions
- Enhance viability and ease of cell culturing of ES cells
- Increase maintenance of pluripotency without serum and feeder cells



Mouse ES cells grown in ESGRO®-2i medium express the pluripotency markers Oct-4, Sox-2 and SSEA1 (left to right).

Product Description	Units	Cat. No.
ESGRO®-2i Supplement Kit (1000x)	1 kit per 500 mL medium	ESG1121
	1 kit per 5 L medium	ESG1120
ESGRO®-2i Medium	100 mL	SF016-100
	200 mL	SF016-200

References

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RESGRO™ Cell Culture Medium

EMD Millipore's RESGRO™ culture medium is a complete medium that can be used to complement traditional murine ES cell culture methods. In contrast to routine ES cell culture, RESGRO™ culture medium is recommended for a number of specialized applications.

Murine ES Cell Derivation

RESGRO™ culture medium enables the efficient derivation and maintenance of ES cell lines from several inbred mouse strains, including certain strains that were previously considered to be non-permissive for ES cell derivation. A recent study demonstrated that RESGRO™ medium allowed the derivation of ES cell lines from inbred strains other than 129. These strains include FVB, a strain previously considered to be non-permissive for ES cell derivation, as well as C57BL/6N, BALB/c, 129/SvEv, and DBA/2N.

Rescue of Established ES Cell Lines

RESGRO™ culture medium has the capacity to rescue traditional ES cell lines that have started drifting and either generate low percentage chimeras or have lost germline transmission capability. Differentiation, which is present in the ES cells but not visible with traditional medium, will become recognizable when using RESGRO™ culture medium. After two passages, a clear difference is seen between differentiated and undifferentiated ES cells, at which time the undifferentiated cells can be removed by sub-cloning.

ES Cell Line	Medium* & Method used	No. of Embryos Transferred	No. of Pups Born	No. of Chimeras Born	Percentage Chimerism
C57BL/6 Knockout clone	Traditional medium Blastocyte injection	50	8	0	0
C57BL/6 Knockout clone	RESGRO™ medium Blastocyte injection	96	38	19	2 died
					2% - 1
					5% - 3
					10% - 4
					20% - 1
					30% - 2
					60% - 1
					70% - 3
					80% - 2

Table 1.

Improved efficiency of murine ES cell lines using RESGRO™ Culture Medium.

Mouse Strain	Blastocysts Cultured (n)	Established ES Cell Lines (n)	Established ES Cell Lines (%)	No. Germline Competent ES Cell Lines/ No. ES Cell Lines Cultured
C57BL/6N	35	18	51	10/11
FVB/N	20	8	40	6/9
BALB/c	34	15	44	7/7
129SvEv	10	6	60	4/4
DBA-2/N	34	13	38	3/3

Table 2.

Efficiency of ES cell derivation and germline competence with RESGRO™ Culture Medium.

Product Description	Qty/Pk	Cat. No.
RESGRO™ Culture Medium	250 mL	SCM001
RESGRO™ Culture Medium	500 mL	SCM002

*Traditional medium: basal medium supplemented with FBS and LIF.

Mouse Embryonic Stem Cell Lines

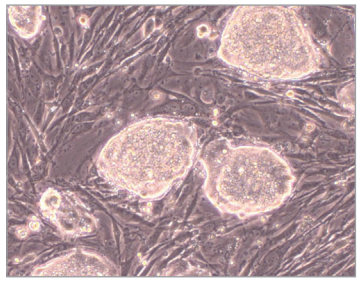
EMD Millipore offers a wide selection of mouse ES cells derived from mice of different genetic backgrounds. The PluriStem® range of cells were derived from inbred strains of mice and are useful for modeling genetic diversity in directed differentiation studies and drug screening bioassays. These lines have high targeting efficiencies and have achieved germline transmission through multiple experiments. Our B6-White™ murine ES cell line is a C57BL/6 tyrc- 2J albino line that allows for rapid coat-color determination of successful chimerism in the C57BL/6 mouse strain. These cells allow for the efficient generation of gene-targeted mice in a pure B6 genetic background, thus providing more experimental flexibility.

Two week-old chimeric mice generated from targeted PluriStem® B6-White ES cells injected into host C57BL/6 blastocysts. Germline transmission from the first litter was obtained.

Product Description	Qty/Pk	Cat. No.
B6-White (C57BL/6 tyrc- 2J) Mouse ES Cell Line	2 vials, 2.5 x 10^6 cells ea.	SCR011
EmbryoMax® Embryonic Stem Cell Line – strain 129/SVEV, passage 11	2 vials, 2.5 x 10^6 cells ea.	CMTI-1
EmbryoMax® Embryonic Stem Cell Line – strain C57BL/6, passage 11	2 vials, 2.5 x 10^6 cells ea.	CMTI-2
PluriStem® 129S6/SvEv Mouse ES Cell Line	2 vials, 2.5 x 10^6 cells ea.	SCR012
PluriStem® C57BL/6N Mouse ES Cell Line, passage 9	2 vials, 2.5 x 10^6 cells ea.	SCC050
PluriStem® DBA/2 Mouse ES Cell Line, passage 9	2 vials, 2.5 x 10^6 cells ea.	SCC054
PluriStem® C3H Mouse ES Cell Line, passage 9	2 vials, 2.5 x 10^6 cells ea.	SCC055
MilliTrace® Constitutive GFP Reporter Mouse Embryonic Stem Cell Kit	1 kit	SCR082
MilliTrace® Nanog GFP Reporter Mouse Embryonic Stem Cell Kit	1 kit	SCR089

Primary Mouse Embryonic Fibroblasts (PMEF)

The EmbryoMax® range of PMEF cells provides researchers with a convenient solution for ES cell culture by eliminating the need for time-consuming feeder cell isolation and preparation. Derived from day 13 embryos, these cells are supplied frozen at passage three (2 populations doublings per passage) in five-vial packs. Each vial contains approximately 5-6 x 10^6 fibroblasts. Several varieties are available, including actively dividing, growth-arrested (mitomycin-C treated), and drug-resistant feeder cells.



EmbryoMax® C57BL/6 mouse ES cells (CMTI-2) cultured on a PMEF feeder layer (PMEF-NL).

Product Description	Treatment	Passage	Qty/Pk	Cat. No.
EmbryoMax® Primary Mouse Embryo Fibroblasts Strain CF1	Not Treated	P1	1 vial, 1 x 10^6 cells ea.	PMEF-CFL-P1
	Not Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-CFL
	Mitomycin-C Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-CF
	Irradiated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-CFX
EmbryoMax® Primary Mouse Embryo Fibroblasts Neo Resistant	Not Treated	P1	1 vial, 1 x 10^6 cells ea.	PMEF-NL-P1
	Not Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-NL
	Mitomycin-C Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-N
	Irradiated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-NX
EmbryoMax® Primary Mouse Embryo Fibroblasts Hygro Resistant	Not Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-HL
	Mitomycin-C Treated	P3	5 vials, 5-6 x 10^6 cells ea.	PMEF-H

Genetic Manipulation

GeneJuice® Transfection Reagent

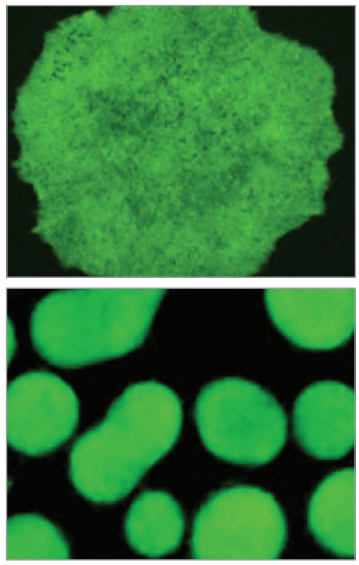
GeneJuice® Transfection Reagent is a proprietary non-lipid based polyamine solution optimized for maximal transfection efficiency, ease of use, and minimal cytotoxicity. This transfection reagent is a superior alternative to a wide variety of other techniques including calcium phosphate coprecipitation, electroporation, microinjection, biolistic particle delivery, and complex formation with DEAE-dextran and liposomes.

Features

- Highly efficient DNA transfer for both stable and transient transfections
- Minimal cellular toxicity
- Compatible with both serum-containing and serum-free media
- Ideal for high-throughput transfection in a multiwell plate format

Benefits

- One reagent, one tube for a wide variety of cell types and applications
- Leads to higher protein expression levels
- Simplifies protocol by eliminating media changes
- Flexibility to accommodate your experimental setup



Stably eGFP-expressing iPSC colonies with ubiquitous eGFP expression derived using GeneJuice® transfection reagent (top). Stably transfected iPSCs retained constitutive eGFP expression during embryoid body differentiation (bottom).

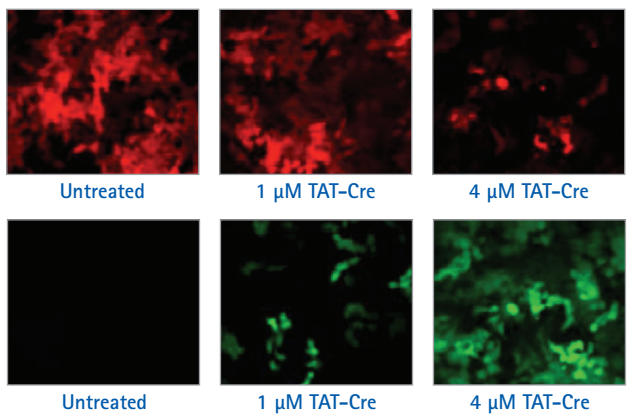
Product Description	Qty/Pk	Cat. No.
GeneJuice® Transfection Reagent	0.3 mL	70967-5
	1 mL	70967-3
	5x1 mL	70967-6
	10x1 mL	70967-4

Tat-Cre Recombinase

Cre Recombinase is an enzyme from bacteriophage P1 that catalyzes the site-specific recombination between two DNA recognition sites termed loxP sites. EMD Millipore's TAT-CRE Recombinase is a recombinant cell-permeant fusion protein consisting of a basic protein translocation peptide derived from HIV-TAT (TAT), and a nuclear localization sequence (NLS).

A.

B.

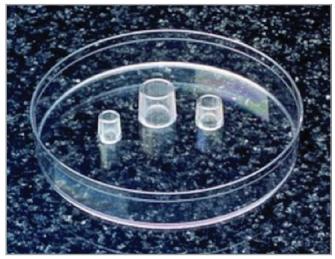


Each lot of TAT-CRE is quality control tested for protein purity and for transduction and recombination activities. A HEK293T cell line stably expressing a double fluorescent reporter construct was used to monitor Cre recombination (A). Cells express dsRed2 before Cre-recombination. Addition of TAT-CRE mediates recombination and results in expression of the eGFP, by deleting the LoxP-flanking dsRed2 gene. Maximal eGFP expression was achieved when 4 µM TAT-Cre was used to treat the cells overnight (B).

Cloning Cylinders

Individual colonies of transfected mouse embryonic stem cells can be isolated and picked from a plate containing many clones. Isolated clones can be dissociated and passaged free from surrounding cells to ensure reproducible results.

Product Description	Cat. No.
Cloning Cylinder, 8 mm x 8 mm diameter	TR-1004
Cloning Cylinder, 10 mm x 10 mm diameter	TR-1005

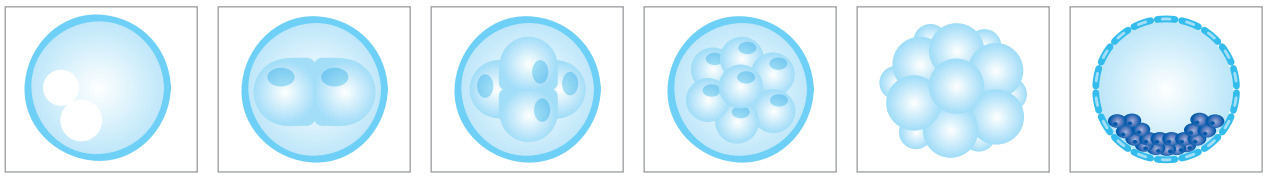


Mouse Embryo Handling and Culture

EmbryoMax® Mouse Embryo Media – Liquid Kits

To enable embryo collection, manipulation, and transfer techniques, EMD Millipore offers a wide selection of mouse embryo media and reagents, including M-2, modified M16, FHM and proprietary KSOM media formulations. Our media are tested on mouse embryos and manufactured using the highest quality raw materials available. Both liquid and powder EmbryoMax® media are available for many classic formulations.

Stages of Embryo Development



Zygote
Mouse Day 0

2-cell
Day 1

4-cell
Day 1-2

Multi-cell
Day 2

Morula
Day 2-3

Blastocyst
Day 3-4

Embryo Handling and Manipulation (HEPES-based media formulations)

M2: To maintain embryos in healthy state during their collection and handling, the M2 media was derived. There are two critical factors in M2 medium that make it suitable for embryo culture outside the CO_2 incubator for extended period of time. The first is its buffering capacity. Upon exposure to air, media that contain only sodium bicarbonate ($NaHCO_3$), such as M16, will quickly become alkaline, whereas HEPES-containing media will not. As a modified Krebs-Ringer solution, M2 contains both HEPES and bicarbonate. The second factor is the energy source. Preimplantation embryos do not utilize glucose efficiently. In this regard, M2 also contains pyruvate and lactate – both can be utilized by the preimplantation embryos.

FHM: With a function similar to M2, the FHM medium is a modification of KSOM where part of bicarbonate is replaced with HEPES buffer. FHM is typically used as an embryo medium for washing and handling embryos outside the CO_2 incubators. Embryo Culture and Development (Bicarbonate-based media formulations)

Embryo Culture and Development (Bicarbonate-based formulations)

KSOM: Advanced media formulation developed using the simplex optimization procedure by Lawitts and Buggers (1991,1992). KSOM media allows outbred zygotes to overcome the two-cell block and supports *in vitro* and *in vivo* development of various mouse strains. KSOM allows for higher rates of cell division and produces higher yields of blastocyst development.

M16: Classic embryo culture media based on modified Krebs-Ringer bicarbonate solution.

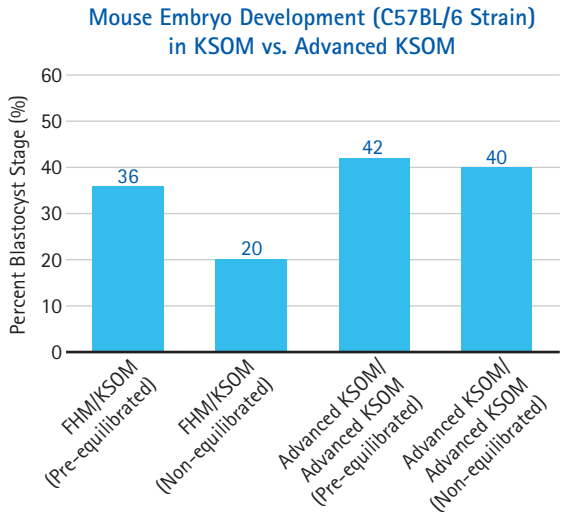
NEW PRODUCT FOCUS

EmbryoMax® Advanced KSOM Embryo Medium

Advanced KSOM is a new uniquely buffered modified version of the original KSOM formulation that enables:

- One medium for both embryo handling in atmospheric conditions and culturing in a CO_2 Incubator.
- Higher frequency of blastocyst formation across multiple mice strains
- No medium pre-equilibration step required

Product Description	Cat. No.
EmbryoMax® Advanced KSOM Embryo Medium	MR-101-D



Product Description	Qty/Pk	Cat. No.
Liquid Kits		
Acidic Tyrode's Solution, for removal of zonae	50 mL	MR-004-D
CZB Medium with phenol red	50 mL	MR-019-D
FHM HEPES Buffered Medium (1X), liquid, with phenol red	50 mL	MR-024-D
FHM HEPES Buffered Medium (1X), liquid, without phenol red	50 mL	MR-025-D
FHM HEPES Buffered Medium with phenol red & hyaluronidase	10 mL	MR-056-F
FHM HEPES Buffered Medium without phenol red & BSA	50 mL	MR-122-D
Human Tubal Fluid (HTF) (1X), liquid, for mouse IVF	50 mL	MR-070-D
KSOM, with 1/2 amino acids, glucose, and phenol red	50 mL	MR-121-D
KSOM, with 1/2 amino acids and glucose	50 mL	MR-106-D
KSOM, with 1/2 amino acids and glucose, without BSA	50 mL	MR-107-D
M2 Medium (1X), liquid, with phenol red	50 mL	MR-015-D
M2 Medium (1X), liquid, with phenol red and hyaluronidase	10 mL	MR-051-F
Modified Dulbecco's Phosphate Buffered Saline, with BSA and phenol red	100 mL	MR-006-C
Modified M16 Medium (1X), witho ut phenol red	50 mL	MR-010-D
Modified M16 Medium (1X), liquid, without phenol red	50 mL	MR-016-D
Powder Kits		
KSOM Embryo Culture Powder (1X), without phenol red	5 x 50 mL	MR-020P-5D
KSOM Embryo Culture Powder (1X), without phenol red	5 x 10 mL	MR-020P-5F
KSOM Embryo Culture Powder (1X), without phenol red	1 x 50 mL	MR-020P-D
M2 Medium (1X), powdered, with phenol red	5 x 50 mL	MR-015P-5D
M2 Medium (1X), powdered, with phenol red	5 x 10 mL	MR-015P-5F
M2 Medium (1X), powdered, with phenol red	1 x 50 mL	MR-015P-D
Modified M16 Medium (1X), powdered, without phenol red	5 x 50 mL	MR-010P-5D
Modified M16 Medium (1X), powdered, without phenol red	5 x 10 mL	MR-010P-5F
Modified M16 Medium (1X), powdered, without phenol red	1 x 50 mL	MR-010P-D

Genotyping

KOD Xtreme™ Hot Start DNA Polymerase

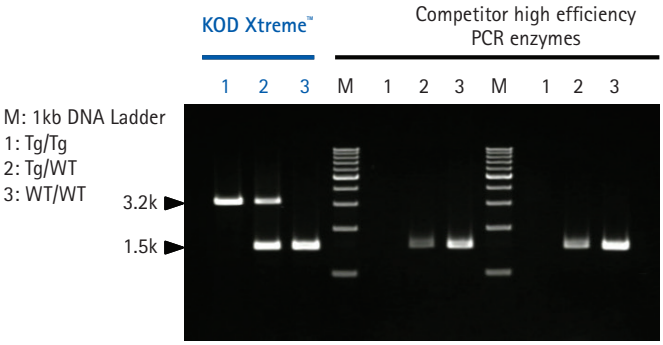
DNA Amplification from Challenging Crude Samples with Minimal Processing

The KOD Xtreme™ Hot Start DNA Polymerase is your enzyme of choice for the most challenging PCR situations including: DNA amplification from crude samples such as mouse tail tip lysates, high GC content, or repeat sequences (T/A) which can inhibit or bias PCR amplification data.

Features

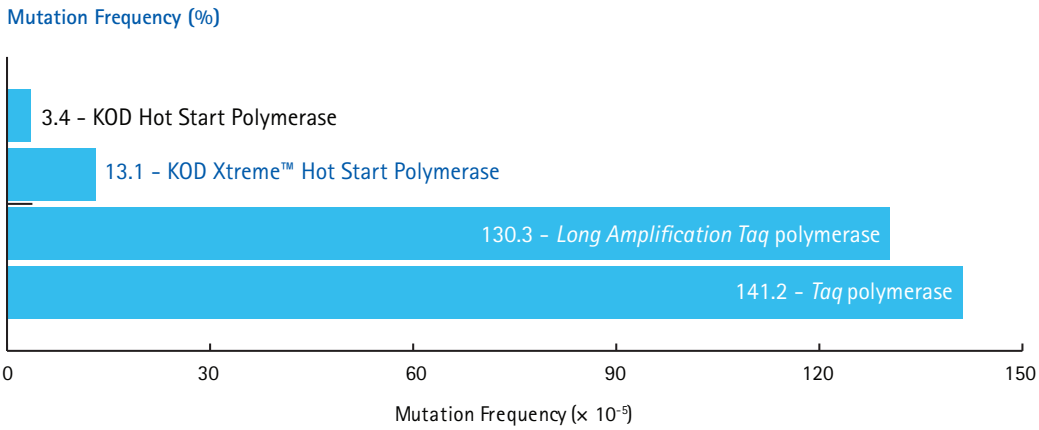
- Optimized for PCR success against complex crude samples and with minimal processing
- Efficiently amplifies up to 90% GC-content templates
- 10x higher fidelity than Taq blends
- Amplifies genomic targets up to 24kb
- Amplifies plasmid/phage targets up to 40kb
- Eliminate mispriming or primer-dimer formation
- Convenient ambient temperature setup compatible with automation

Amplify DNA from crude tissue lysate with minimal processing to save time and minimize cost while maintaining quality data.



The targeted locus genes were amplified with various PCR enzymes using mouse tail lysates prepared by alkaline lysis*. KOD Xtreme™ Hot Start DNA polymerase successfully amplified both targets (Tg and WT).

Ultra high fidelity of KOD Hot Start and KOD Xtreme™ Hot Start DNA Polymerases during long PCR amplification.



Amplification from Mouse Tail Using Rapid Crude Lysate Preparation with Alkaline Lysis Method; Optimized Protocol for Microtube or 96-well Plate Format

STEP 1. Cut ~3 mm piece of mouse tail
STEP 2. Transfer to microfuge tube (or 96-well plate) containing 180 µL of 50 mM NaOH and vortex well
STEP 3. Incubate at 95 °C for 10 min on a thermocycler
STEP 4. Add 20 µL of 1 M Tris-HCl (pH 8.0) and vortex well (take care to avoid contamination)
STEP 5. Centrifuge lysate at 12,000 RPM for 5 mins
STEP 6. Add 0.5-2 µL of lysate to PCR reaction (50 µL) as below

Component	Volume	Final Concentration
2x Xtreme™ Buffer	25 µL	1x
dNTPs (2 mM each)	10 µL	0.4 mM
PCR Grade Water	X µL	
Sense (5') Primer -10 µM (10 pmol/ µL)	1.5 µL	0.3 µM
Antisense (3') Primer-10 µM (10 pmol/µL)	1.5 µL	0.3 µM
KOD Xtreme™ Hot Start DNA Polymerase (1.0 U/µL)	1.0 µL	
Total Volume	**50 µL**	

STEP 7. Example Thermocycling Conditions

Two-step Cycling	
1. Polymerase Activation	94 °C for 2 min
2. Denaturation	98 °C for 10 s
3. Annealing and Extension	68 °C for 1 min/kb

Repeat step 2-3 for 30 cycles.

Product Description	Qty/Pk	Cat. No.
KOD Xtreme™ Hot Start DNA Polymerase	200 U	71975-3
Components: 1 x 200 U Polymerase 3 x 1.7 mL 2x Xtreme™ Buffer 2 x 1 mL dNTPs (2 mM each)		

Superovulation Hormones

Superovulation has been used in the production of transgenic mice since the late 1980s to artificially induce ovulation of large numbers of oocytes from limited numbers of female mice. Superovulation facilitates the generation of genetically engineered mice and reduces the number of animals used. The hormones Pregnant Mare Serum Gonadotropin (PMSG) and human chorionic gonadotrophin (hCG) are the two main inducers of superovulation in mice. EMD Millipore is a leading supplier of Calbiochem® brand PMSG and hCG to induce superovulation of mice.

Product Description	Cat. No.
Gonadotropin, Pregnant Mare Serum	367222
Chorionic Gonadotropin, Human Urine	230734

Other EmbryoMax® Media and Reagents

EMD Millipore offers a broad range of cell culture media and reagents for the mouse ES cell culture workflow. Our EmbryoMax® line of ES cell-qualified reagents provides researchers with convenient and cost-effective solutions for the reliable culture of ES cells. These products negate the need for researchers to screen lots of media, reagents, and serum, thus delivering significant cost and time savings.

Product Description	Qty/Pk	Cat. No.
ES Cell Qualified Fetal Bovine Serum		
EmbryoMax® ES Cell Qualified Fetal Bovine Serum, US origin	500 mL	ES-009-B
EmbryoMax® ES Cell Qualified Fetal Bovine Serum, US origin	100 mL	ES-009-C
Basal Media		
EmbryoMax® DMEM (1X), low bicarbonate formulation, with 4,500 mg/L glucose, 2.25g/L sodium bicarbonate & L-glutamine, without sodium pyruvate	500 mL	SLM-120-B
EmbryoMax® DMEM (1X), liquid, with 4,500 mg/L glucose, without L-glutamine & sodium pyruvate	1 L	SLM-021-A
EmbryoMax® DMEM (1X), liquid, with 4,500 mg/L glucose, without L-glutamine & sodium pyruvate	500 mL	SLM-021-B
EmbryoMax® DMEM (1X), liquid, low bicarbonate formulation, with 4,500 mg/L glucose, 2.25 g/L sodium bicarbonate, without L-glutamine & sodium pyruvate	500 mL	SLM-220-B
EmbryoMax® DMEM (1X), liquid, low bicarbonate formulation, with 4,500 mg/L glucose, 2.25 g/L sodium bicarbonate, without L-glutamine & sodium pyruvate	400 mL	SLM-220-M
Reagents & Media Supplements		
EmbryoMax® 0.1% Gelatin Solution	500 mL	ES-006-B
EmbryoMax® 2-Mercaptoethanol (100X)	20 mL	ES-007-E
EmbryoMax® Electroporation Buffer	50 mL	ES-003-D
EmbryoMax® Filtered Light Mineral Oil	100 mL	ES-005-C
EmbryoMax® Filtered Silicon Oil	100 mL	ES-004-C
EmbryoMax® L-Glutamine Solution (100X), 200 mM	100 mL	TMS-002-C
EmbryoMax® 1M HEPES Buffer Solution, Liquid	100 mL	TMS-003-C
EmbryoMax® MEM, Non-Essential Amino Acids (100X)	100 mL	TMS-001-C
EmbryoMax® Nucleosides (100X)	50 mL	ES-008-D
EmbryoMax® Penicillin-Streptomycin Solution	100 mL	TMS-AB2-C
EmbryoMax® DPBS (1X)	1 L	BSS-1005-A
EmbryoMax® DPBS (1X)	500 mL	BSS-1005-B
EmbryoMax® DPBS (1X), without Ca^{2+} or Mg^{2+}	1 L	BSS-1006-A
EmbryoMax® DPBS (1X), without Ca^{2+} or Mg^{2+}	500 mL	BSS-1006-B
EmbryoMax® DPBS (10X), without Ca^{2+} or Mg^{2+}	500 mL	BSS-2010-B
EmbryoMax® DPBS (10X), with Ca^{2+} and Mg^{2+}	500 mL	BSS-6010-B
EmbryoMax® Ultra Pure Water, sterile	1 L	TMS-006-A
EmbryoMax® Ultra Pure Water, sterile	500 mL	TMS-006-B
EmbryoMax® Ultra Pure Water, sterile	100 mL	TMS-006-C

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