KnockOut™ SR

Catalog Numbers 10828028, 10828010

Pub. No. MAN0007312 Rev. 6.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ KnockOut™ Serum Replacement (KnockOut™ SR) is a more defined, FBS-free formulation designed to directly replace fetal bovine serum (FBS) in supporting the growth and derivation of human and other mammalian embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) on fibroblast feeder cells. For human ESCs, KnockOut™ SR also supports cryopreservation, embryoid body formation, and *in vitro* differentiation studies. For mouse ESCs, KnockOut™ SR can replace FBS in media used for blastocyst injection, embryo culture, electroporation or cationic lipid transfection, clone selection, cryopreservation, derivation of new ESC lines, embryoid body formation, and in vitro differentiation studies. By providing the documentation required for regulatory review, KnockOut™ SR enables you to reduce your burden in qualifying reagents when transitioning from research applications to clinical applications. Each container is sterile filtered.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
KnockOut™ Serum Replacement	10828010 10828028	100 mL 500 mL	–20°C to –5°C. Protect from light.	18 months

[1] Shelf-Life duration is determined from Date of Manufacture.

Culture conditions

Cells: Mouse, human and other mammalian ESCs and iPSCs

Culture type: Adherent co–culture

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO_2 in air. Ensure proper gas exchange and minimize exposure of cultures to light.

Procedural guidelines

- Refer to **thermofisher.com** for applications and protocols using KnockOut™ SR, including detailed protocols for mouse or human ESC/iPSC culture.
- KnockOut™ SR cannot be used as a replacement for FBS in the plating of feeder cells.
- KnockOut™ SR does not contain trypsin inhibitors. Therefore, trypsin must be removed or inactivated when culturing ESCs or iPSCs in medium containing KnockOut™ SR.
- Due to the variability observed between pluripotent stem cell (iPSC and ESC) lines, we recommend that you test different lots of KnockOut™ SR to optimize your culture conditions.

Guidelines to prepare media

- Thaw KnockOut™ SR overnight at 4°C.
- Alternately, thaw KnockOut™ SR in a 37°C water bath with frequent gentle swirling to expedite thawing.

IMPORTANT! Do not heat-inactivate.

- Occasionally flocculent material may be observed while thawing. This material will go into solution with gentle swirling at 37°C. Minimize dwell time in waterbath.
- KnockOut™ SR is stable for up to 4 weeks at 2°C to 8°C, protected from light.
- Working volumes can be aliquoted and stored at –20°C to –5°C. Thaw aliquots as needed. Avoid additional freeze-thaw cycles.
- If using traditional DMEM for mouse ESCs/iPSCs, we recommend using KnockOut™ DMEM instead.

Guidelines for use

- Complete medium is stable for 10 days when stored in the dark at 2°C to 8°C.
- Avoid repeated warming and chilling of the complete medium. Warm only the volume required for that day's use.
- For best results, pre-equilibrate complete medium to temperature (37°C) and gases (5% CO_2 in humidified air) before use.

- If lyophilized, reconstitute basic Fibroblast Growth Factor (bFGF) and LIF Recombinant Mouse Protein, ESC-Qualified to stock concentrations of 10 µg/mL in 0.1% bovine serum albumin.
- GlutaMAX™ Supplement may be substituted with L-glutamine at the same molar concentration.
- Supplement complete medium with fresh 0.1 mM 2-Mercaptoethanol immediately prior to equilibrating medium to temperature and gases.
- If desired, antibiotics may be used.

Prepare complete media

Prepare complete media for human ESCs and iPSCs (Table 1) or mouse ESCs and iPSCs (Table 2) cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs).

Table 1 Media for human ESCs/iPSCs

Reagent	Stock conc.	Final conc.	For 100 mL
DMEM/F-12, GlutaMAX™ Supplement	–	1X	79 mL
KnockOut™ SR	–	20%	20 mL
NEAA[1]	10 mM	0.1 mM	1 mL
bFGF[2]	10 µg/mL	4 ng/mL	40 µL
2-Mercaptoethanol[3]	55 mM	0.1 mM	182 µL

[1] Non-Essential Amino Acids
[2] basic Fibroblast Growth Factor
[3] Add immediately prior to use.

Table 2 Media for mouse ESCs/iPSCs

Reagent	Stock conc.	Final conc.	For 100 mL
KnockOut™ DMEM	–	1X	83 mL
KnockOut™ SR	–	15%	15 mL
NEAA[1]	10 mM	0.1 mM	1 mL
GlutaMAX™ Supplement	200 mM	2 mM	1 mL
LIF Recombinant Mouse Protein, ESC-Qualified	10 µg/mL	10 ng/mL	100 µL
2-Mercaptoethanol[2]	55 mM	0.1 mM	182 µL

[1] Non-Essential Amino Acids
[2] Add immediately prior to use.

Note: KnockOut™ SR cannot be used as a replacement for FBS in the plating of feeder cells. While the formulation contains sufficient factors to allow plating of ESCs and iPSCs, fibroblasts have an increased need for undefined attachment factors and will not adequately attach in this formulation. However, once plated, the feeder cell layer will remain attached to the plates when placed into media containing KnockOut™ SR.

Dissociate mouse ESC/iPSCs

For cell dissociation of mouse ESCs and iPSCs, we highly recommend using StemPro™ Accutase™ Cell Dissociation Reagent , as described in the detailed online protocol. Trypsin may also be used. When dissociating ESC/iPSC clones with trypsin following electroporation and selection, use soybean trypsin inhibitor to quench trypsin activity.

1. Prepare a sterile 5 mg/mL solution of soybean trypsin inhibitor in DPBS, no calcium, no magnesium.
2. Trypsinize clones and then add one-tenth the volume of soybean trypsin inhibitor to the trypsinized ESCs/iPSCs.
3. Transfer cells to KnockOut™ SR supplemented medium and replate.

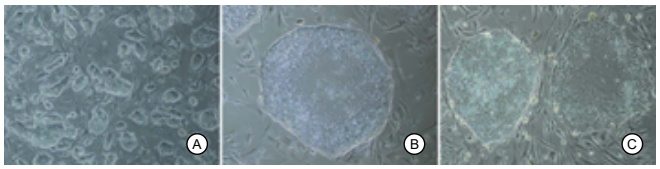
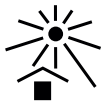


Figure 1 Morphology of ESCs and iPSCs on MEFs

Morphology of C57BL/6 mouse ESCs A), H9 human ESCs B), and human iPSCs C) cultured on MEFs in media containing KnockOut™ SR.

Explanation of symbols and warnings

				
Temperature Limitation	Manufacturer	Batch Code	Use By	Catalog Number
				
Caution, consult accompanying documents	Consult instructions for use	Keep away from light	Sterilized using aseptic processing techniques	Read Safety Data Sheet

Related products

Item	Source
KnockOut™ DMEM (1X)	10829018
KnockOut™ DMEM/F-12 (1X)	12660012
DMEM/F-12, GlutaMAX™ Supplement (1X)	10565018
KnockOut™ SR–Multi-Species	A3181501 A3181502
KnockOut™ SR Xeno-Free	A1099201
KnockOut™ ESC/iPSC Media Kit	A1412901
CF1 Mouse Embryonic Fibroblasts, Irradiated	A34181
L-Glutamine, 200 mM (100X), liquid	25030081 25030024[1]
GlutaMAX™-1, 200 mM (100X)	35050061 35050038[1]
FGF-basic, Recombinant Human Protein	13256029
LIF Recombinant Mouse Protein, ESC-Qualified	A35933
MEM Non-Essential Amino Acids Solution (100X)	11140076 11140068
2-Mercaptoethanol (100X)	31350010[1]
StemPro™ Accutase™ Cell Dissociation Reagent	A1110501
TrypLE™ Express Enzyme (1X), no phenol red	12604013
Trypsin-EDTA, (1X)	25300054
Trypsin Inhibitor, Soybean	17075029
DPBS, no calcium, no magnesium	14190144 [1]

[1] For European Customers Only

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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The information in this guide is subject to change without notice.