

# KnockOut™ SR

## Description

KnockOut™ Serum Replacement (KnockOut™ SR) is a defined, serum-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) as well as induced pluripotent stem cells (iPSCs) when cultured on fibroblast feeder cells. KnockOut™ SR also supports cryopreservation, embryoid body formation, and *in vitro* differentiation studies. For murine 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. Each container is sterile filtered.

Product	Catalog no.	Amount	Storage	Shelf life*
KnockOut™ Serum Replacement	10828-010	100 mL	-20°C to -5°C; Protect from light	18 months
	10828-028	500 mL		18 months

\* Shelf Life duration is determined from Date of Manufacture.

## Intended use

For human *ex vivo* tissue and cell culture processing applications. Caution: When used as a medical device, Federal Law restricts this device to sale by or on the order of a physician.

## Important information

- Refer to [www.lifetechnologies.com/stemcells](http://www.lifetechnologies.com/stemcells) for detailed protocols and new applications using KnockOut™ SR.
- 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 in KnockOut™ SR-containing medium.
- 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.

## Safety information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Culture conditions

**Media:** The use of KnockOut™ DMEM or KnockOut™ DMEM/F12 is recommended for optimal growth and maintenance of pluripotent ESC/iPSCs.

**Cells:** human and other mammalian ESCs, iPSCs, and mESCs

**Culture type:** adherent co-culture

**Temperature range:** 36°C to 38°C

**Incubator atmosphere:** Humidified atmosphere of 4–6% CO<sub>2</sub> in air. Ensure proper gas exchange and minimize exposure of cultures to light.

## Prepare media

To thaw KnockOut™ SR, place at 4°C overnight. Alternatively, KnockOut™ SR can be thawed in a 37°C water bath with frequent gentle swirling to expedite thawing. **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, KnockOut™ DMEM is recommended and if using traditional DMEM/F12, KnockOut™ DMEM/F12 is recommended.

## Use

The following tables are for the preparation of complete media for human ESCs and iPSCs (Table 1) or murine ESCs and iPSCs (Table 2) cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs). 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<sub>2</sub> in humidified air) before use.

- Reconstitute basic Fibroblast Growth Factor (bFGF) and Leukemia Inhibitory Factor (LIF) to stock concentrations of 10 µg/mL in Dulbecco's Phosphate Buffered Saline (D-PBS) with 0.1% bovine serum albumin.
- GlutaMAX™-I may be substituted for L-glutamine at the same molar concentration.
- Supplement complete medium with 0.1 mM fresh 2-mercaptoethanol immediately prior to equilibrating medium to temperature and gases.
- If desired, antibiotics may be used.

**Table 1** Media for human ESCs/iPSCs

Reagents	Stock conc.	Final conc.	For 100 mL
KnockOut™ DMEM or KnockOut™ DMEM/F12	—	1X	78 mL
KnockOut™ SR	—	20%	20 mL
NEAA*	10 mM	0.1 mM	1 mL
bFGF	10 µg/mL	4 ng/mL	40 µL
L-glutamine	200 mM	2 mM	1 mL
2-Mercaptoethanol**	55 mM	0.1 mM	182 µL

\* Non-Essential Amino Acids \*\* Add immediately prior to use

**Table 2** Media for murine ESCs/iPSCs

Reagents	Stock conc.	Final conc.	For 100 mL
KnockOut™ DMEM	—	1X	83 mL
KnockOut™ SR	—	15%	15 mL
NEAA*	10 mM	0.1 mM	1 mL
L-glutamine	200 mM	2 mM	1 mL
LIF	10 µg/mL	10 ng/mL	100 µL
2-Mercaptoethanol**	55 mM	0.1 mM	182 µL

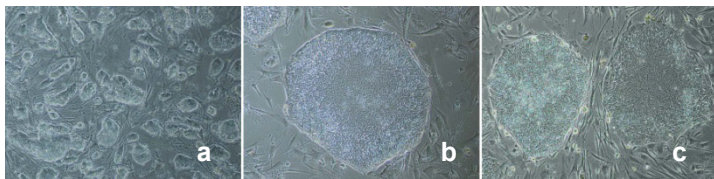
\* Non-Essential Amino Acids \*\* Add immediately prior to use

**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.

### Cell dissociation of murine ESCs

For cell dissociation of mESCs we recommend StemPro® Accutase®, but trypsin may also be used. When dissociating ESC 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 D-PBS without calcium or magnesium.
2. Trypsinize clones and then add one-tenth the volume of soybean trypsin inhibitor to the trypsinized ESCs.
3. Transfer cells to KnockOut™ SR supplemented medium and replat.



**Figure 1** Morphology of C57BL/6 murine ESCs (a), H9 human ESCs (b), and human iPSCs (c) cultured on MEFs in media containing KnockOut™ SR.

### Related products

Product	Catalog no.
KnockOut™ DMEM (1X), liquid	10829
KnockOut™ DMEM/F-12 (1X), liquid	12660
KnockOut™ SR XenoFree,	A10992
KnockOut™ ESC/iPSC Media Kit	A1412901
Gibco® Mouse (ICR) Inactivated Embryonic Fibroblasts	A24903
L-Glutamine, 200 mM (100X), liquid	25030
GlutaMAX™-I, 200 mM (100X), liquid	35050
FGF-basic, Recombinant Human, lyophilized powder	13256
LIF, Recombinant Human, lyophilized powder	PHC9484
MEM Non-Essential Amino Acids (100X), liquid	11140
2-Mercaptoethanol (1000X), liquid	21985 & 31350*
StemPro® Accutase® Cell Dissociation Reagent	A11105
TrypLE™ Express (1X), liquid, without Phenol Red	12563
Trypsin-EDTA, (1X)	25300
Trypsin Inhibitor, Soybean	17075
Dulbecco's Phosphate Buffered Saline, without calcium and magnesium	14190

\* For European Customers Only

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### Important licensing information

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

For further assistance, email [techsupport@lifetech.com](mailto:techsupport@lifetech.com).

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