

## KNOCKOUT™ SR

### Serum Replacement for ESCs / iPSCs

GIBCO® KNOCKOUT SR is a serum-free formulation designed to directly replace fetal bovine serum (FBS) and is used to support the growth of undifferentiated pluripotent stem cells in culture.

KNOCKOUT SR can be used for routine maintenance and derivation of human and non-human primate embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). It 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.

Description	Cat. No.	Size
KNOCKOUT™ SR	10828-010	100mL
	10828-028	500mL

### 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.

### Precautions

#### DO NOT HEAT-INACTIVATE.

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.

KNOCKOUT SR **does not contain trypsin inhibitors.** Therefore, trypsin must be removed or inactivated when culturing ESCs in KNOCKOUT SR containing medium.

### Storage and Handling

Store at -5° to -20°C, Protect from light.

To thaw KNOCKOUT SR, place at 2 to 8°C overnight. If the bottle is not completely thawed by the next day, place the bottle in a 37°C water bath and continue thawing until the ice is gone and the supplement is clear. Gently swirl the bottle occasionally while in the water bath. Alternatively, KNOCKOUT SR can be thawed in a 37°C water bath, as long as the bottle is swirled frequently throughout the thawing process.

Occasionally some flocculent material may be observed while thawing, but this material will go into solution with gentle swirling at 37°C. Minimize dwell time in waterbath.

Store thawed KNOCKOUT SR at 2 to 8°C in the dark for up to 4 weeks, or aliquot into working volumes and store at -5 to -20°C. Thaw aliquots as needed. Avoid additional freeze-thaw cycles.

### Shelf Life

12 months

### Physical Conditions

Standard physical conditions for human or murine embryonic stem cells grown in KNOCKOUT SR complete medium are 36 to 38°C in a humidified atmosphere of 4 to 6% CO<sub>2</sub> in air. Ensure that proper gas exchange is achieved in culture vessels. Avoid overexposure of cultures to light.

### Medium Preparation

The following tables are for the preparation of complete media for human (Table 1) or murine (Table 2) ESCs and iPSCs cultured on mitotically inactivated mouse embryonic fibroblasts (MEFs). Complete medium is stable for 10 days when stored in the dark at 2 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 (36 to 38°C) and gases (4 to 6% CO<sub>2</sub> in humidified air) before use.

**Table 1: Medium for human ESCs/iPSCs**

	Stock Conc.	Catalog Number	Final Conc.	For 100 mL
KNOCKOUT DMEM or KNOCKOUT DMEM/F12	-	10829 or 12660	1X	78 mL
KNOCKOUT SR	-	10828	20%	20 mL
NEAA	10 mM	11140	0.1 mM	1.0 mL
bFGF	10 µg/mL	13256	4 ng/mL	40 µl
L-glutamine	200 mM	25030	2 mM	1.0 mL
2-Mercaptoethanol	55 mM	21985	0.1 mM	182 µl

**Table 2: Medium for murine ESCs/iPSCs**

	Stock Conc.	Catalog Number	Final Conc.	For 100 mL
KNOCKOUT DMEM	-	10829	1X	83 mL
KNOCKOUT SR	-	10828	15%	15 mL
NEAA	10 mM	11140	0.1 mM	1.0 mL
L-glutamine	200 mM	25030	2 mM	1.0 mL
LIF	10 µg/mL	PHC9464	10 ng/mL	100 µl
2-Mercaptoethanol	55 mM	21985	0.1 mM	182 µl

- It is recommended to add fresh 0.1mM 2-Mercaptoethanol to complete medium just prior to that day's use.
- Reconstitute bFGF and LIF to a stock concentration of 10 µg/mL in PBS with 0.1% bovine serum albumin.
- If desired, antibiotics can be used.
- GlutaMAX™ (Cat. No. 35050) may be substituted for L-Glutamine at the same molar concentration.

**Note:** The use of KNOCKOUT DMEM or KNOCKOUT DMEM/F12 is recommended for the best ESC/iPSC results. If using traditional DMEM, KNOCKOUT DMEM is recommended and if using traditional DMEM/F12, KNOCKOUT DMEM/F12 is recommended.

## Use:

KNOCKOUT SR may be used for the growth and maintenance of undifferentiated murine and human ESCs/iPSCs at the same FBS concentration currently used (typically 15-20%).

Refer to [www.invitrogen.com/stemcells](http://www.invitrogen.com/stemcells) for detailed protocols and new applications with KNOCKOUT SR:

[GIBCO® Mouse Embryonic Fibroblasts](#)

[GIBCO® Mouse \(129\) Embryonic Stem Cells](#)

[GIBCO® Mouse \(129\) Embryonic Stem Cells with GFP](#)

[GIBCO® Mouse \(C57BL/6\) Embryonic Stem Cells](#)

[GIBCO® Mouse \(C57BL/6\) Embryonic Stem Cells with GFP](#)

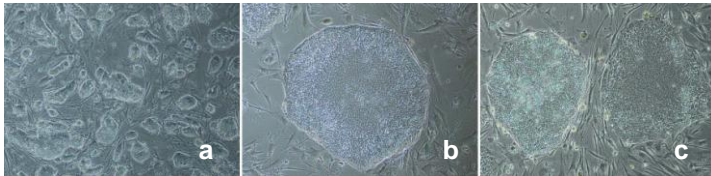
[Human Embryonic Stem Cell Starter Kit](#)

[Induced Pluripotent Stem Cells](#)

## Cell Dissociation of Murine ESCs

For cell dissociation, StemPro® Accutase® (Cat. No. A11105) is recommended, but trypsin may also be used.

When dissociating ESC clones with trypsin following electroporation and selection, the use of soybean trypsin inhibitor (Cat. No. 17075) is recommended to quench trypsin activity. Prepare a sterile 5 mg/ml solution of soybean trypsin inhibitor in DPBS. Trypsinize clones and then add one-tenth the volume of soybean trypsin inhibitor to the trypsinized ESCs. 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

KNOCKOUT™ DMEM (1X), liquid (10829)

KNOCKOUT™ SR XenoFree, (A10992)

KNOCKOUT™ DMEM/F-12 (1X), liquid (12660)

L-Glutamine, 200 mM (100X), liquid (25030)

Basic Fibroblast Growth Factor (bFGF), Human, Recombinant (10 µg), lyophilized powder (13256)

MEM Non-Essential Amino Acids (100X), liquid (11140)

2-Mercaptoethanol (1000X), liquid (21985 & 31350\*)

StemPro Accutase, (A11105)

Trypsin Inhibitor, soybean (17075)

Dulbecco's Phosphate Buffered Saline (DPBS) without calcium, magnesium, or phenol red (1X), liquid (14190)

Recombinant Human LIF, lyophilized powder (PHC9464)

\*For European Customers Only

## Technical Support

For additional product and technical information, such as Material Safety Data Sheets (MSDS), Certificate of Analysis, etc, please visit our website at [www.invitrogen.com](http://www.invitrogen.com). For further assistance, please email our Technical Support team at [Techsupport@Invitrogen.com](mailto:Techsupport@Invitrogen.com).

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KNOCKOUT™ is a trademark of Life Technologies Corporation. Accutase® is a registered trademark of Innovative Cell Technologies, Inc.

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