


KnockOut™ SR–Multi-Species

Catalog Numbers A3181501, A3181502

Pub. No. MAN0015933 Rev. 2.0

 **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–Multi-Species (KnockOut™ SR–Multi-Species) is a more defined, fetal bovine serum (FBS)-free medium supplement that supports the growth of mouse, human and other mammalian embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) cultured on fibroblast feeder cells. For mouse and human, KnockOut™ SR–Multi-Species also supports reprogramming, embryoid body (EB) formation, *in vitro* differentiation studies, cryopreservation and ESC derivation. In addition, KnockOut™ SR–Multi-Species can replace FBS in media used for mouse blastocyst injection, embryo culture, electroporation or transfection, as well as clone selection. With the same formulation as the original KnockOut™ Serum Replacement, KnockOut™ SR–Multi-Species is designed to directly replace fetal bovine serum (FBS) in existing protocols.

Contents and storage

| Contents | Cat. No. | Amount | Storage | Shelf life ^[1] |
|---|----------|--------|------------------------------------|---------------------------|
| KnockOut™ Serum Replacement–Multi-Species | A3181501 | 100 mL | –20°C to –5°C. Protect from light. | 18 months |
| | A3181502 | 500 mL | | |

^[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% CO₂ 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–Multi-Species, including detailed protocols for mouse or human ESC/iPSC culture.
- KnockOut™ SR–Multi-Species cannot be used as a replacement for FBS in the plating of feeder cells.
- KnockOut™ SR–Multi-Species does not contain trypsin inhibitors. Therefore, trypsin must be removed or inactivated when culturing ESCs or iPSCs in medium containing KnockOut™ SR–Multi-Species.
- Due to the variability observed between pluripotent stem cell (iPSC and ESC) lines, we recommend that you test different lots of KnockOut™ SR–Multi-Species to optimize your culture conditions.

Guidelines to prepare media

- Thaw KnockOut™ SR–Multi-Species overnight at 4°C.
- Alternately, thaw KnockOut™ SR–Multi-Species 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–Multi-Species 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% CO₂ 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–Multi-Species | – | 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–Multi-Species | – | 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–Multi-Species 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–Multi-Species.

Dissociate mouse ESCs/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–Multi-Species supplemented medium and replate.

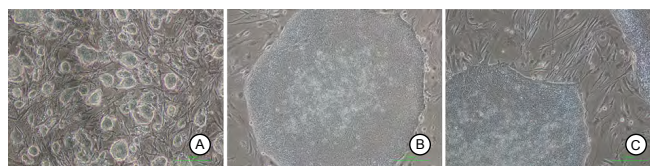












Figure 1 Morphology of ESCs and iPSCs on MEFs

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

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 | 10828010 10828028 |
| 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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