CTS™ KnockOut™ SR XenoFree

Catalog Numbers A1099201 and A1099202

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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

CTS™ KnockOut™ SR XenoFree enables the growth and expansion of human embryonic stem cells (hESC) in a cell culture medium containing only human-derived or human recombinant proteins, to facilitate hESC research. CTS™ KnockOut™ SR XenoFree does not contain bovine or other non-human, animal-derived components. Besides hESC culture expansion and maintenance, CTS™ KnockOut™ SR XenoFree can be used for hESC cryopreservation, hESC and induced pluripotent stem cell (iPSC) derivation, and hESC differentiation studies. Each container is sterile filtered.

Contents and storage

Contents	Cat. No.	Amount	Storage	Shelf life[1]
CTS™ KnockOut™ SR XenoFree	A1099201	100 mL	–20°C to –5°C. Protect from light.	18 months
	A1099202	500 mL		

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

Safety information

Human origin materials are non-reactive (donor level) for anti-HIV 1 & 2, anti-HCV, and HBsAg. Handle in accordance with established bio-safety practices.

Culture conditions

Media: Complete CTS™ KnockOut™ SR XenoFree Medium

Cell type: hESC or iPSC

Culture type: Adherent

Recommended culture vessels: T-Flasks

Temperature range: 36°C to 38°C

Incubator atmosphere: Humidified atmosphere of 4–6% CO_2 in air. For best results, pre-equilibrate complete medium to temperature (37°C) and gases (5% CO_2 in humidified air) before use. Ensure proper gas exchange and avoid overexposure of cultures to light.

Note: Procedures detailed in the following sections are for cultures in T-75 culture flasks (75 cm^2). Volumes should be adjusted accordingly for desired vessel size.

Important information

To thaw CTS™ KnockOut™ SR XenoFree, place at 2°C to 8°C overnight. Alternatively, CTS™ KnockOut™ SR XenoFree 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.
- CTS™ KnockOut™ SR XenoFree 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.

Refer to **www.thermofisher.com/stemcells** for detailed protocols and new applications using KnockOut™ products.

CTS™ KnockOut™ SR XenoFree cannot be used as a replacement for FBS in the plating of feeder cells.

CTS™ KnockOut™ SR XenoFree does not contain trypsin inhibitors. Therefore, trypsin must be removed or inactivated when culturing ESCs in CTS™ KnockOut™ SR XenoFree-containing medium.

Prepare media

- Prepare complete media for human ESCs and iPSCs as outlined in Table 1.

- Complete medium is stable for at least 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.
- Reconstitute basic Fibroblast Growth Factor (bFGF) to a stock concentration of 10 µg/mL in Dulbecco's Phosphate Buffered Saline (DPBS).

Table 1 Media for human ESCs/iPSCs

Reagents	Stock conc.	Cat. No.	Final Conc.	For 100 mL
CTS™ KnockOut™ DMEM	—	A12861	1X	82.75 mL
CTS™ KnockOut™ SR XenoFree	—	A10992	15%	15 mL
CTS™ GlutaMAX™-I Supplement	200 mM	A1286001	2 mM	1 mL
MEM Non-Essential Amino Acids Solution (100X)	10 mM	11140	0.1 mM	1 mL
bFGF	10 µg/mL	13256	8 ng/mL	80 µL
2-Mercaptoethanol[1]	55 mM	21985	0.1 mM	182 µL

[1] It is recommended to add fresh 2-Mercaptoethanol (only to the volume required for that day's use) immediately prior to use of CTS™ KnockOut™ SR XenoFree complete medium.

Prepare wash medium

Dilute 2.5 mL CTS™ KnockOut™ SR XenoFree in 97.5 mL CTS™ KnockOut™ DMEM. Wash medium is stable at 2°C to 8°C for up to 2 weeks.

Procedural guidelines

- Cultures may be grown in CTS™ KnockOut™ SR XenoFree complete medium using either feeder cells or feeder-free conditions.
- For XenoFree culture using human foreskin fibroblast (HFF) feeder cells, tissue-culture treated vessels can be coated with CELLStart™ Substrate humanized substrate prior to plating HFF in complete medium.
- Once HFF feeder cells have attached and spread (generally 8 hours to overnight), hESCs can be plated on the HFF vessels as desired (see Figure 1).
- Research indicates that CTS™ KnockOut™ SR XenoFree and CELLStart™ Substrate will also support feeder-free growth of hESCs when supplemented with bFGF and additional growth factors.

For more information contact our Technical Support Team at **thermofisher.com/askaquestion**.

Recover cryopreserved hESCs with CTS™ KnockOut™ SR XenoFree

1. Rapidly thaw (<1 minute) frozen vial of cells in a 37°C water bath, until a small frozen piece remains in the vial.
2. Decontaminate vial with 70% isopropyl alcohol.
3. Aseptically transfer the entire contents of the vial into a 15-mL conical tube.
4. Dropwise, add 3 mL pre-warmed CTS™ KnockOut™ SR XenoFree complete medium to the conical tube containing thawed hESC.
5. Rinse the vial with 1–2 mL fresh, pre-warmed CTS™ KnockOut™ SR XenoFree complete medium and add to the same conical tube.
6. Pellet cells by centrifuging at 200 × g at room temperature for 2 minutes. Aspirate and discard the supernatant without disturbing the cell pellet.
7. Gently "flick" the tube to fully dislodge the cell pellet from the tube bottom.
8. Add the desired volume of pre-equilibrated CTS™ KnockOut™ SR XenoFree complete medium to the hESC pellet.

   Do not triturate cells.
9. Gently invert the conical tube containing hESCs to mix cells. Using a pipet, transfer the cells to a prepared feeder-containing CELLStart™ Substrate-coated cell culture vessel (see CELLStart™ Substrate coating of culture vessels).
10. Place vessel in a 37°C incubator with a humidified atmosphere of 5% CO2 in air. Carefully swirl vessel in a back and forth and then a left and right pattern to evenly distribute hESC.
11. Exchange spent media with fresh CTS™ KnockOut™ SR XenoFree complete medium 24 hours post-thaw and daily thereafter, until approximately 70–80% confluent.

Guidelines for hESc adaptation to CTS™ KnockOut™ SR XenoFree

Different hESC lines will behave differently in CTS™ KnockOut™ SR XenoFree medium and optimal growth conditions must be determined for each application.

- Starter cultures should be of high quality, be 70–80% confluent, and contain no differentiated hESCs.
- **Feeder cultures**: The best adaptation results will be obtained when the parent hESC culture has been maintained in traditional KnockOut™ SR on either murine embryonic fibroblast (MEF) or HFF feeder cells prior to adapting to CTS™ KnockOut™ SR XenoFree.

- **Feeder-free cultures**: The best adaptation results will be obtained when the parent hESC culture has been maintained in traditional MEF-conditioned medium (MEF-CM) prior to adapting to CTS™ KnockOut™ SR XenoFree.
- Make a frozen bank of cells in control medium prior to adaptation.
- Maintain a "backup" culture in control medium throughout hESC adaptation to CTS™ KnockOut™ SR XenoFree.
- The hESCs should be nearing confluence (70–80%) at the time of passage. If hESCs are passaged at low confluency or when overgrown, hESCs will differentiate.
- **IMPORTANT!** If seeded too low, hESCs will differentiate. The hESC cultures must be fluid-changed daily for optimal performance.

Direct adaptation

If hESCs are passaged directly into CTS™ KnockOut™ SR XenoFree complete medium, a 1:2 split ratio is suggested for the first 3 passages.

- To increase the chances of successful adaptation, seed one plate directly in CTS™ KnockOut™ SR XenoFree complete medium and two in MEF-CM control medium. Fluid-change the CTS™ KnockOut™ SR XenoFree plate and one control plate with CTS™ KnockOut™ SR XenoFree complete medium that day and daily thereafter. At the second passage, seed both plates directly in CTS™ KnockOut™ SR XenoFree complete medium at 1:2.

Sequential adaptation

Best results may be obtained by gradually adapting hESCs to CTS™ KnockOut™ SR XenoFree.

- Passage 1: 75% control medium + 25% CTS™ KnockOut™ SR XenoFree complete medium.
- Passage 2: 50% control medium + 50% CTS™ KnockOut™ SR XenoFree complete medium.
- Passage 3: 25% control medium + 75% CTS™ KnockOut™ SR XenoFree complete medium.
- Passage 4 and thereafter: 100% CTS™ KnockOut™ SR XenoFree complete medium.
- If the hESC line is difficult to adapt, a further level of caution can be taken by maintaining a culture in each prior passage medium while starting the next level of adaptation. For example, when passaging the 25/75 control medium/ CTS™ KnockOut™ SR XenoFree culture (Passage 3, above), hESCs can be passaged into both 100% CTS™ KnockOut™ SR XenoFree medium AND 25/75 medium. If the 100% culture does poorly, adaptation can be resumed using the backup 25/75 culture.

Coat culture vessels with CELLStart™ Substrate

1. Dilute CELLStart™ Substrate 1:50 in CTS™ DPBS with calcium, magnesium . Pipet gently to mix. DO NOT VORTEX.
2. Add diluted CELLStart™ Substrate to culture plates at a final volume per surface area of 78 µL/cm^2. Refer to table for respective culture container:

Culture vessel	Surface area (cm^2)	Volume of diluted CELLStart™ Substrate
60-mm plate	28.25	2.25 mL/ plate
6-well plate	9.6	750 µL/ well
12-well plate	3.2	250 µL/ well
24-well plate	2.0	160 µL/ well

3. Incubate at 37°C in a humidified atmosphere containing 5% CO_2 in air, for 1–2 hours.
4. After incubation, remove coated vessels from the incubator. For immediate use, place vessels at room temperature. For use the next day, carefully wrap vessels containing diluted CELLStart™ Substrate with Parafilm™ laboratory film, and store at 2°C to 8°C.

Plate feeder cells

1. Immediately before use, remove all CELLStart™ Substrate diluent from the vessel. It is not necessary to rinse vessels following removal of CELLStart™ Substrate.
2. If growing cells in a feeder-free system proceed with plating hESCs on the CELLStart™ Substrate-coated vessel at the desired density (see Passaging hESCs Using CTS™ KnockOut™ SR XenoFree).
3. If growing hESCs on feeder cells, harvest inactive fibroblast feeder cells (e.g., HFF) as normal, resuspending feeders in CTS™ KnockOut™ SR XenoFree Complete Medium.

   Note: Addition of bFGF is not essential for plating feeders
4. Plate inactive feeders on CELLStart™ Substrate -coated vessels at the desired density.
5. Place feeder-seeded vessels in a 37°C incubator, with a humidified atmosphere of 5% CO_2 in air. Carefully swirl vessel in a back and forth and then a left and right pattern to evenly distribute fibroblast feeder cells.
6. Incubate overnight to enable feeder attachment and spreading. Vessels are now ready to receive hESCs and can be used for up to one week.

Passage hESCs with CTS™ KnockOut™ SR XenoFree

CTS™ KnockOut™ SR XenoFree can be used in place of KnockOut™ SR for the maintenance of hESCs in feeder-containing or feeder-free culture systems. To maintain a xeno-free culture system, CTS™ TrypLE™ Select Enzyme is recommended for cell dissociation.

1. Observe stock culture vessel (hESCs growing in current medium formulation or in CTS™ KnockOut™ SR XenoFree Complete Medium) under the microscope and confirm that the cells are ready to be subcultured (70–80% confluent).

2. Cut out and remove any differentiated hESC colonies prior to passaging the culture. A 22 gauge 1½″ needle attached to a syringe works well for removing differentiated hESCs.

3. Pre-warm the required volumes of CTS™ TrypLE™ Select Enzyme and Wash Medium to 37°C, and pre-equilibrate the required volume of CTS™ KnockOut™ SR XenoFree Complete Medium to temperature and gases before use. Minimize dwell time.

4. Aspirate and discard the spent medium.

5. Rinse hESCs twice with CTS™ DPBS without calcium chloride, without magnesium chloride

6. Add warm CTS™ TrypLE™ Select Enzyme to the culture vessel (1 mL/ 60-mm dish). Swirl vessel to coat the entire cell surface.

7. Place in 37°C incubator for 2–3 minutes (less time may be required for feeder-free hESC dissociation).

8. Remove vessel from the incubator. Gently tap the sides of the dish to dislodge cells.

9. Transfer cells to a sterile 15-mL conical tube.

10. Rinse dish twice with pre-warmed Wash Medium, gently "spraying off" any cells that haven't detached, and pool with cells in tube.

IMPORTANT! Do not triturate.

11. Pellet cells by centrifugation at 200 × g for 2 minutes at room temperature. Aspirate and discard the supernatant without disturbing the cell pellet.

12. Gently "flick" the tube to fully dislodge the cell pellet from the tube bottom.

13. Gently resuspend the cells in pre-equilibrated complete medium using a 2-mL or 5-mL serological pipette.

IMPORTANT! Do not triturate.

14. Transfer cells to a fresh feeder-plated or CELLStart™ Substrate -coated vessel at the desired cell ratio or seeding density. A 1:2 split is recommended during adaptation, or 8 × 10^4 hESC/cm^2. For routine maintenance, cells can be split at 1:4 – 1:8, or 4 × 10^4 hESC/cm^2 using CTS™ KnockOut™ SR XenoFree Complete Medium. Adjust densities as needed to suit your particular hESC line.

15. Incubate at 37°C in a humidified atmosphere containing 5% CO_2 in air. Carefully swirl vessel in a back and forth and then a left and right pattern to evenly distribute hESCs.

16. Gently fluid-change culture the next day to remove cell debris and to provide fresh nutrients, and daily thereafter.

17. Observe cells daily and passage by the above protocol whenever required (approximately every 3–5 days).

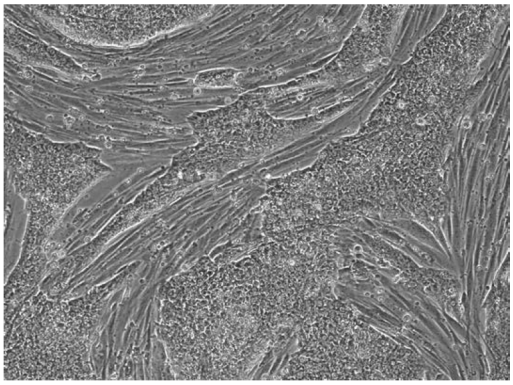

Figure 1 Xeno-Free Growth of hESCs on Feeders

BG01v morphology when cultured in 15% CTS™ KnockOut™ SR XenoFree on human foreskin fibroblasts (HFF) attached with CELLStart™ Substrate; passage 4.

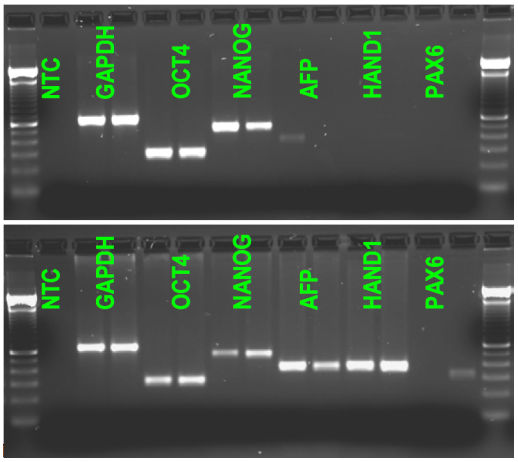

Figure 2 Maintenance of Pluripotency using CTS™ KnockOut™ SR XenoFree

Following 10 passages in either KnockOut™ SR (left lane) or CTS™ KnockOut™ SR XenoFree (right lane) on HFF attached with CELLStart™ Substrate, BG01v gene expression was examined (top). Gene expression of embryoid bodies generated from the same P10 BG01v/HFF cultures (bottom).

Cryopreservation of hESCs with CTS™ KnockOut™ SR XenoFree

- Prepare cryopreservation medium by supplementing CTS™ KnockOut™ SR XenoFree Complete Medium with an additional 10% CTS™ KnockOut™ SR XenoFree (to yield a final concentration of 25%) and 10% Dimethyl Sulfoxide (DMSO) cryoprotectant.
- Expect some cell death at recovery, and freeze hESCs at a higher density than would normally be passaged (if cells are routinely passaged at a 1:5 dilution, a 1:3 or 1:4 dilution is recommended).
- Follow the protocol for Passaging hESCs Using CTS™ KnockOut™ SR XenoFree through step 12, gently resuspend the cell pellet with cryopreservation medium without triturating.
- While vialing, invert the capped hESC tube routinely to mix the cells. For best results, hESC vials should be cryopreserved using a controlled rate freezing device. Transfer frozen cells to liquid nitrogen (vapor phase); storage at –200°C to –125°C is recommended.

Gibco™ XenoFree Media and reagents include products that may contain discrete proteins, bulk protein fractions or recombinant proteins of human or non-animal origin. They may also contain proteins, hydrolysates, or components of unknown composition of human or non-animal origin.

Related products

Item	Source
CELLStart™ Substrate	A10142
CTS™ DPBS with calcium, magnesium	A12858
CTS™ DPBS without calcium chloride, without magnesium chloride	A12856
CTS™ KnockOut™ DMEM	A12861
CTS™ GlutaMAX™-I Supplement	A1286001
CTS™ TrypLE™ Select Enzyme	A12859
CTS™ KnockOut™ SR XenoFree	A30209
L-Glutamine (200 mM)	A2916801
FGF-Basic Full Length CTS™ Recombinant Human Protein	CTP0261
MEM Non-Essential Amino Acids Solution (100X)	11140050
2-Mercaptoethanol	21985023
Gentamicin (50 mg/mL)	15750
Collagenase Type IV powder	17104
Penicillin-Streptomycin	15070063
Trypan Blue Stain	15250
Countess™ II Automated Cell Counter	AMQAX1000

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