Human Pluripotent Stem Cell Growth Medium DXF

PromoCell

Instruction Manual

Product	Size	Catalog Number
hPSC Growth Medium DXF	500 ml	C-28060

Recommended for

- Human Pluripotent Stem Cells (hPSC)

Product Description

The PromoCell hPSC Growth Medium DXF (hPSC-GM DXF) was designed for the feeder-free undifferentiated expansion of human pluripotent stem cells, e.g. hiPS and hESC. The formulation is not only chemically defined / xeno-free, but also completely excludes substances purified from human or animal origin. In addition, the medium works with growth factor concentrations in the lower physiological range. The optimal culture environment allows for a well-controlled culture process, consistent and reproducible performance, robust support of pluripotency and improved cloning efficiency.

In combination with the extracellular matrix hPSC-ECM DXF and the hPSC Dissociation Buffer DXF PromoCell provides a defined and xeno-free complete culture system. The hPSC-ECM DXF is a defined and xeno-free extracellular matrix of recombinant origin. The non-enzymatic chemically defined and xeno-free hPSC Dissociation Buffer DXF was designed for gentle but efficient subculture of hPSC. It supports clump as well as single cell passage. For detailed information, please see www.promocell.com/application-notes.

Supplementation Details

PromoCell hPSC-GM DXF contains all growth factors and supplements except attachment- and spreading factors (see paragraph II.1 of the hPSC Culture Protocol below). PromoCell hPSC-GM DXF does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO_2.

Preparation of the Supplemented Medium for Use

Thaw the SupplementMix at 15 to 25°C. Make sure there is no precipitate left. If necessary warm the SupplementMix in your hands and mix vigorously until all components have solubilized. Aseptically mix the supplement solution by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

Note: Use the completely supplemented hPSC Growth Medium DXF within 10 days. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 2 to 8°C protected from light.

For detailed information and illustrated step-by-step protocols, please see www.promocell.com/application-notes.

Use aseptic techniques and a laminar flow bench.

hPSC Culture Protocol

I. Materials and media/solutions

- hPSC Growth Medium DXF (C-28060)
- hPSC Dissociation Buffer DXF (C-41322)
- hPSC-ECM DXF, 20x conc. (C-43070)
- Dulbecco's PBS, w/o Ca^{++}/Mg^{++} (C-40232)

Optionally needed for thawing of frozen hPSC or single cell subculture:
- Y-27632 (PK-CA577-1596-1) *or alternatively*
- Thiazovivin (PK-CA577-1681-1)

II. Initiation of the culture

The protocol describes the steps for switching existing cultures to the PromoCell hPSC Growth Medium DXF. Ensure that cultures are in good condition. Pluripotency should be ≥90% for optimal results.

1. Coat the culture vessel with ECM

Dilute the thawed ECM stock solution of hPSC-ECM DXF 1:20 with Dulbecco's PBS, w/o Ca^{++}/Mg^{++}. Use 100 µl per cm^2 of culture surface to coat the closed tissue culture vessel with the diluted ECM solution and leave for 2 hours at room temperature. Make sure that the ECM solution covers the complete vessel. If not to be used immediately, the sealed vessel may be stored for up to 7 days at 2 to 8°C for later use. Aspirate the ECM solution just before seeding the cells. Diluted ECM solution may be stored for up to 2 weeks at 2 to 8°C protected from light.

Note: For best results PromoCell recommends using of the hPSC-ECM DXF. However, other established types of appropriate ECM can be used.

2. Prepare the complete hPSC Growth Medium DXF

Thaw the SupplementMix in your hands and mix thoroughly. Make sure there is no precipitate left. Prepare the complete hPSC Growth Medium DXF by adding the thawed SupplementMix aseptically to the Basal Medium. Swirl gently to obtain a homogeneous mixture.

Note: Use the completely supplemented hPSC Growth Medium DXF within 10 days. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 2 to 8°C protected from light.

3. Plate the cells (day 0)

For existing proliferating cultures perform a clump passage as described below in step III. A. Seed the cells in a 1:2 to 1:3 ratio into the ECM coated tissue culture vessel using an appropriate amount of hPSC Growth Medium DXF. For an example, use 2 - 3 ml per well for 6 well plates and 15-25 ml for T-75 flasks.
For cryopreserved cells, add a ROCK-Inhibitor (10 µM Y-27632 or 2 µM Thiazovivin) to the thawing medium as well as to the hPSC Growth Medium DXF.

Note: Adaption of hPSC to the PromoCell hPSC Growth Medium DXF is not necessary. However, allow up to 5 passages to reach full performance.

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4. **Let the cells attach (day 0)**
   After plating let the subcultured cells attach over night (12 - 24 hours).

5. **Medium change (day 1)**
   Perform a medium change: aspirate the medium including non-attached cells, wash once with Dulbecco's PBS, w/o Ca^{++}/Mg^{++} and provide the cells with fresh hPSC Growth Medium DXF (w/o ROCK-Inhibitors).

   Note: Do not add ROCK-Inhibitors.

6. **Cell expansion (day 1+)**
   Perform daily media changes. Let the cells expand until the single colonies touch each other (usually after 4 - 7 days) or colonies show the first signs of differentiation evoked from their large size. Proceed with step III) for subculture of the cells.

   Note: For established hPSC cultures, the hPSC Growth Medium DXF supports extended feeding intervals for up to 48 hours as an exception. However, this is not recommended for the initiation phase of the culture.
   The PromoCell hPSC Growth Medium DXF can rescue differentiating cultures. Perform a 1:1 ratio clump passage (see step III. A) and change medium twice daily until the differentiated cells are lost and cell/colony morphology has normalized.

III. Clump- or single cell subculture of hPSC

A. Clump passage (recommended for routine culture)

1. Aspirate culture medium and wash cells twice with Dulbecco's PBS, w/o Ca^{++}/Mg^{++}.
2. Add 200 - 300 µl/cm² of hPSC Dissociation Buffer DXF and incubate for 5-8 minutes in the incubator at 37°C and 5% CO_2.
   Note: Other established enzymatic dissociation/subculture procedures can also be used.
3. Carefully aspirate the Dissociation Buffer and add 1 - 5 ml of fresh hPSC Growth Medium DXF. Using a serological pipet, flush the colonies away from the surface. Avoid flushing more than 4 - 5 times in order to maintain cell clumps of sufficient size.
4. Dispense cell clumps in new ECM-coated culture vessels with fresh hPSC Growth Medium DXF.
5. Proceed according to the cell attachment step II. 4.

B. Single cell passage

1. Aspirate culture medium and wash cells twice with Dulbecco's PBS, w/o Ca^{++}/Mg^{++}.
2. Add 200 - 300 µl/cm² of hPSC Dissociation Buffer DXF and incubate for 5-8 minutes in the incubator at 37°C and 5% CO_2.
   Note: Other established enzymatic dissociation/subculture procedures can also be used.
3. Aspirate the Dissociation Buffer and replace by 50 - 100 µl/cm² fresh hPSC Dissociation Buffer DXF supplemented with a ROCK-Inhibitor (10 µM Y-27632 or 2 µM Thiazovivin).

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4. Detach the cells by flushing the culture surface several times using a serological pipet. Triturate by pipetting up and down an additional 5-10 times.
5. Spin the single cell suspension (5 min, 200 x g, room temperature).
6. Aspirate and discard the supernatant and resuspend the cells in fresh hPSC Growth Medium DXF supplemented with a ROCK-Inhibitor (10 µM Y-27632 or 2 µM Thiazovivin).
   Note: Alternatively, resuspend the cells in a buffer of choice and use them for your experiments, e.g. immunostaining for flow cytometry analysis.
7. Plate the cells in an ECM-coated vessel and proceed with the attachment according to step II. 4.

Storage and Stability

Store the Basal Medium at 4 to 8°C in the dark, store the SupplementMix at -20°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the SupplementMix to the Basal Medium, the shelf life of the complete medium is 10 days at 4 to 8°C stored in the dark. Do not freeze the complete medium.
For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4 to 8°C and protect from light.

Note: The SupplementMix is delivered thawed and can be frozen after arrival without losing any activity.

Quality Control

All lots of PromoCell hPSC-GM DXF are subjected to comprehensive quality control tests using human pluripotent stem cells. Each lot of PromoCell hPSC-GM DXF is tested for the ability to support the undifferentiated expansion of human pluripotent stem cells by performing a morphology test and an additional pluripotency test: the cells are cultured for three passages with periodic visual inspection for maintenance of the typical morphology and proliferation. In passage four, the cells are stained for the pluripotency marker profile Oct-3/4, SSEA-1 and SSEA-4. Analysis is performed by flow cytometry.
In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

Intended Use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

If you require special media modifications, we offer a Custom Media Service starting at 10 bottles per order. Please ask for details.

Related Products

Product	Size	Catalog Number
hPSC-ECM DXF (20x)	2 ml	C-43070
hPSC Dissociation Buffer DXF	100 ml	C-41322

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