Chondrocytes

PromoCell®

Instruction manual

Product	Size	Catalog number
Human Chondrocytes (HCH)	500,000 cryopreserved cells 500,000 proliferating cells	C-12710 C-12750

Product description

Articular cartilage covers the bones in the major joints of the human body, e.g. knee and hip joints. It is composed of only one specialized cell type, Chondrocytes. Chondrocytes produce and maintain the extracellular matrix of cartilage, i.e. collagen (mostly type II) and proteoglycans. The isolated, differentiated cells express collagen type II; however, during prolonged *in vitro* cultivation, dedifferentiation begins and collagen type II expression declines.

Our Human Chondrocytes (HCH) are produced at our cell culture facility and are available from knee or hip joint cartilage tissue.

Shortly after isolation, all our Human Chondrocytes are cryopreserved using our proprietary, animal-component free, and protein-free cryopreservation medium (for details, please refer to the Certificate of Analysis).

Each cryo vial contains more than 500,000 viable cells after thawing. Thawing and seeding results in passage 2.

Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days in our hands and shipped as growing cultures.

Quality control

We perform rigid quality control tests for each lot of Human Chondrocytes.

The cells are tested for cell morphology, adherence rate, and cell viability. Furthermore, each lot is routinely tested for the capacity to differentiate in 3D spheroids. Growth performance is tested through multiple passages up to 10 population doublings (PD) under culture conditions without antibiotics or antimycotics.

In addition, all cells have been tested for the absence of HIV-1, HIV-2, HBV and HCV, and microbial contaminants (fungi, bacteria, and mycoplasma).

Intended use

Our Human Chondrocytes are for *in vitro* research use only and not for diagnostic or therapeutic procedures.

Warning

Although tested negative for HIV-1, HIV-2, HBV, and HCV, the cells – like all products of human origin – should be handled as potentially infectious. No test procedure can completely guarantee the absence of infectious agents.

Follow appropriate safety precautions!

After delivery, cryopreserved cells should be stored in liquid nitrogen or seeded directly (see page 2). Proliferating cells must be processed immediately (see page 3).

Protocol for cryopreserved cells

Straight after arrival, store the cryopreserved cells in liquid nitrogen, or seed them immediately.

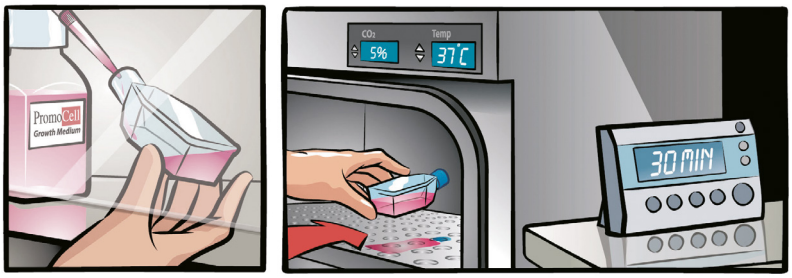
Note: Storage at -80°C is not sufficient for cell preservation and causes irreversible cell damage.

Use aseptic techniques and a laminar flow bench.

1

Prepare the medium

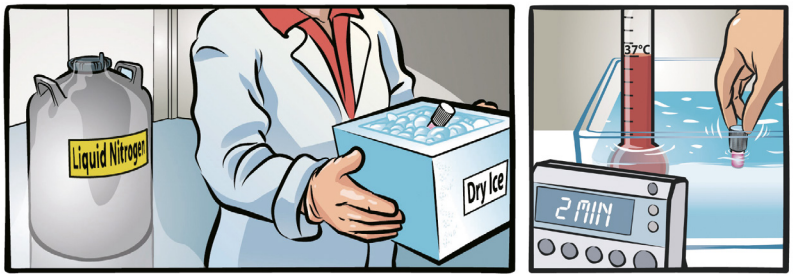
Calculate the required culture surface area according to the plating density (see page 5) and the lot-specific cell numbers stated on the certificate of analysis. Fill the appropriate volume of PromoCell Growth Medium (at least 9 ml per vial of cells) in cell culture vessels. Place the vessels in an incubator (37°C, 5% CO_2) for 30 minutes.



2

Thaw the cells

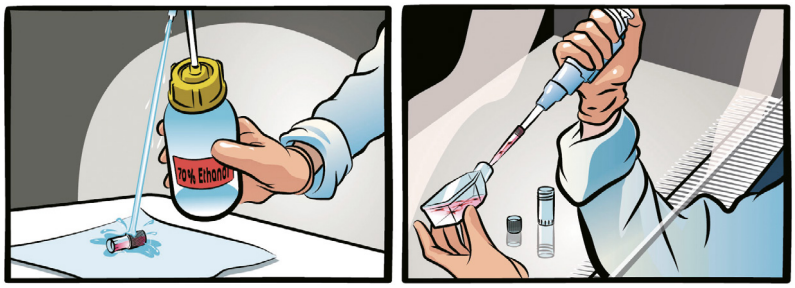
Remove the cryovial from the liquid nitrogen container and immediately place it on dry ice – even for short transportation. Under a laminar flow bench, briefly twist the cap a quarter turn to relieve pressure, then retighten. Immerse the vial in a water bath (37°C) up to the height of the screw cap for 2 minutes. Ensure that no water enters the thread of the screw cap.



3

Disinfect the vial and seed the cells

Thoroughly rinse the cryovial with 70% ethanol under a laminar flow bench. Then, aspirate the excess ethanol from the thread area of the screw cap. Open the vial and transfer the cells to a cell culture vessel containing the pre-warmed medium from step 1.



4

Incubate the cells

Place the vessel in an incubator (37°C, 5% CO_2) for cell attachment. Replace the medium after 16–24 hours and every two to three days thereafter. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70–90% confluency.

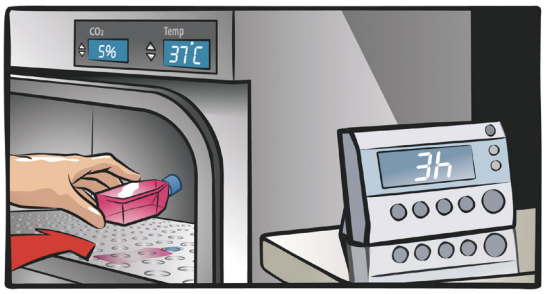
Protocol for proliferating cells

Start immediately after delivery.
Use aseptic techniques and a laminar flow bench.

1

Incubate the cells

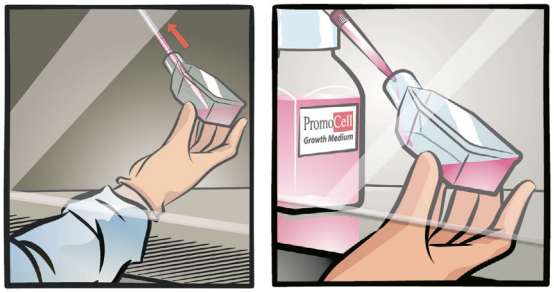
Unpack the culture vessel, do not open the lid, and immediately place it in an incubator (37°C, 5% CO_2) for 3 hours to allow the cells to recover from transportation.



2

Replace the transport medium

Carefully open the vessel, rinse the inner side of the lid with 70% ethanol, and let air dry. Aspirate the transport medium from the vessel. Add 10 ml of the appropriate PromoCell Cell Growth Medium.



3

Check and incubate the cells

Check the cell density. Open the lid half a turn and place the vessel in an incubator (37°C, 5% CO_2). Change the medium every two to three days. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached >70% confluency.

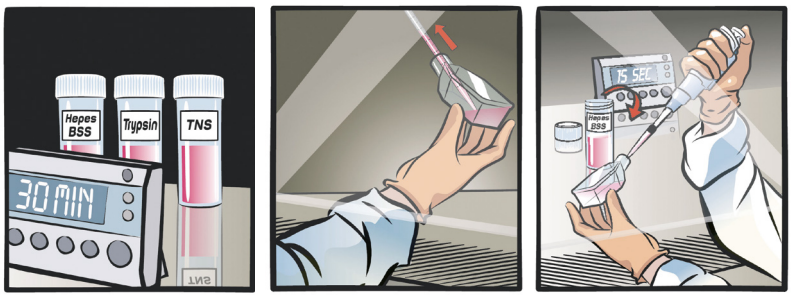
Subcultivation protocol

Use aseptic techniques and a laminar flow bench.

1

Prepare the reagents and wash the cells

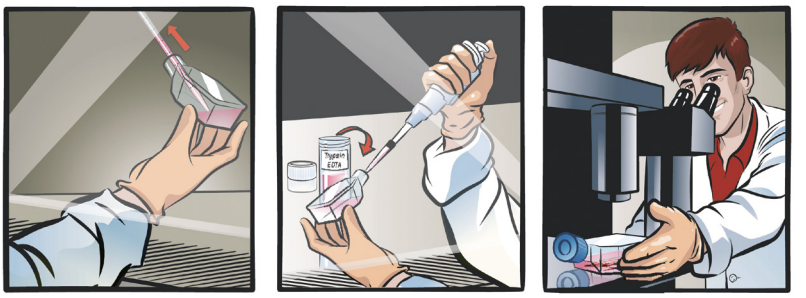
Place the PromoCell DetachKit at room temperature for at least 30 minutes to adjust the temperature of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm² of vessel surface to wash the cells and agitate the vessel carefully for 15 seconds.



2

Detach the cells

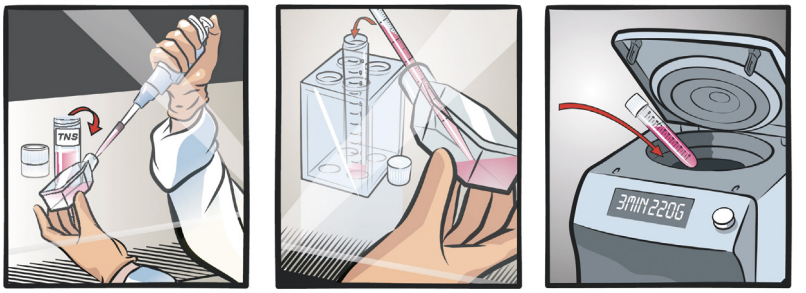
Carefully aspirate the Hepes BSS from the culture vessel. Add 100 µl Trypsin/EDTA Solution per cm² of vessel surface. Note: We recommend detaching the cells at room temperature. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.



3

Neutralize the trypsin and harvest the cells

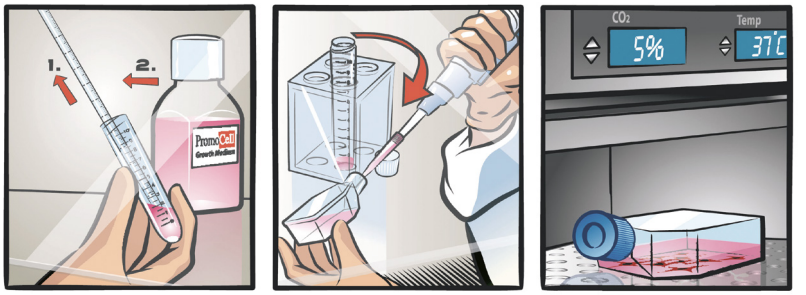
Add 100 µl Trypsin Neutralization Solution per cm² of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. Spin down the cells for 3 minutes at 220 x g.



4

Incubate the cells

Discard the supernatant (step 1), add 1 ml of the appropriate PromoCell Cell Growth Medium (step 2), and resuspend the cells by carefully pipetting up and down. Plate the cells according to the recommended seeding density in new cell culture vessels containing PromoCell Cell Growth Medium prewarmed to 37°C. Place the vessels in an incubator (37°C, 5% CO_2) and change the media every two to three days.



Specifications

Product	Recommended culture media*	Plating density	Passage after thawing	Marker	Population doublings
Human Chondrocytes (HCH)	C-27101	10,000 – 20,000 cells per cm^2	P2	Differentiation tested	> 10

**The catalog numbers in this table are for media in ready-to-use packaging.*

Related products

Product	Size	Catalog number
Chondrocyte Growth Medium (Ready-to-use)	500 ml	C-27101
Chondrocyte Basal Medium	500 ml	C-27111
Chondrocyte Basal Medium, phenol red-free	500 ml	C-27115
Chondrocyte Growth Medium SupplementMix	for 500 ml	C-39635
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM Plus	30 ml 125 ml	C-29920 C-29922
HCH Pellet	1 million cells per pellet	C-14070

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