Preadipocytes

PromoCell

Instruction Manual

Product	Size	Catalog Number
Human White Preadipocytes (HWP) subcutaneous	500,000 cryopreserved cells 500,000 proliferating cells	C-12730 C-12731
Human White Preadipocytes (HWP) visceral	500,000 cryopreserved cells 500,000 proliferating cells	C-12732 C-12733

Product Description

Adipose tissue is crucially involved in energy storage and metabolic homeostasis of the body. Human White Preadipocytes (HWP) are self-renewing progenitors of mature differentiated adipocytes and can be found as a constant subpopulation in adipose tissue throughout adult life. PromoCell offers a range of Human White Preadipocytes produced at PromoCell's cell culture facility from normal human subcutaneous and visceral adipose tissue from different locations of the body (lot specific source information is available on request). Differentiation of HWP into mature adipocytes can be performed using PromoCell Preadipocyte Differentiation Media system (see Instruction Manual "Preadipocyte/Adipocyte Media").

Shortly after isolation, all PromoCell Human White Preadipocytes are cryopreserved at passage 2 (P2) using PromoCell´s proprietary serum-free freezing medium Cryo-SFM. Each cryo vial contains more than 500,000 viable cells after thawing.

Proliferating cell cultures are made from 500,000 cryopreserved cells that have been thawed and cultured for three days at PromoCell.

Quality Control

Rigid quality control tests are performed for each lot of PromoCell Human White Preadipocytes.

They are tested for cell morphology, adherence rate, and cell viability. Growth performance is tested through multiple passages up to 10 population doublings (PD) under culture conditions without antibiotics and antimycotics. Furthermore, each lot of PromoCell Human White Preadipocytes is extensively tested for its capacity to differentiate into mature adipocytes.

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

A detailed certificate of analysis (CoA) for each lot can be downloaded at: www.promocell.com/coa

Intended Use

PromoCell Human White Preadipocytes 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, start immediately with the protocol for cryopreserved cells (see page 2) or the protocol for proliferating cells (see page 3).

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

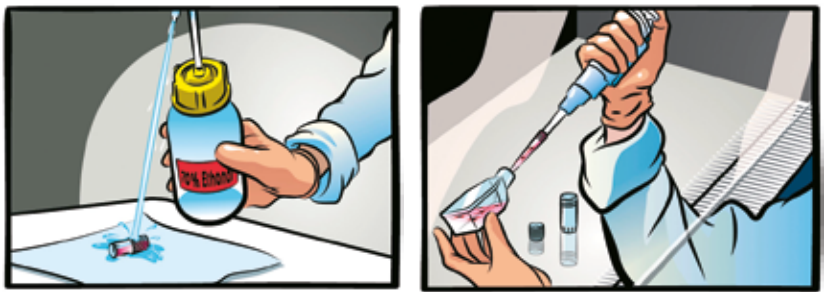
Protocol for Cryopreserved Cells

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.

1. **Prepare the medium**
   Calculate the needed culture surface area according to the plating density (see page 5). 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 re-tighten. Immerse the vial into a water bath (37°C) just up to 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 prewarmed 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. The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 - 90% confluency.

Start immediately after delivery.
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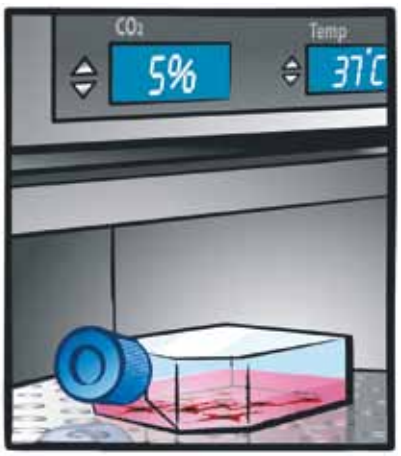
Protocol for Proliferating Cells

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.

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 a half turn and place the vessel in an incubator (37°C, 5% CO_2). The cells should be subcultured, according to the subcultivation protocol (see page 4), once they have reached 70 – 90% confluency.



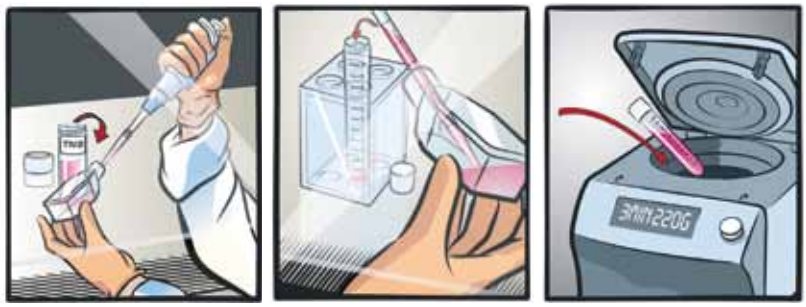
Use aseptic techniques and a laminar flow bench.

Subcultivation Protocol

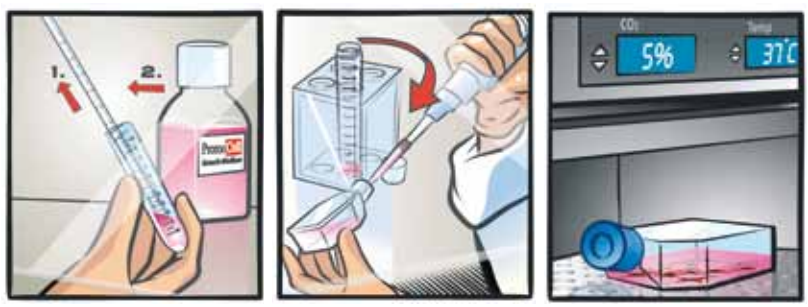
1. **Prepare the reagents and wash the cells**
   Place the PromCell DetachKit at room temperature for at least 30 minutes to adjust the temper ture of the reagents. Carefully aspirate the medium from the culture vessel. Add 100 µl Hepes BSS Solution per cm^2 of vessel surface to wash the cells and agitate 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 cm^2 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).



Specifications

Product	Recommended Culture Media*	Plating Density	Passage after Thawing	Marker	Population Doublings
Human White Preadipocytes (HWP) subcutaneous	C-27410 C-27436 C-27438	5,000 cells per cm²	P2	Differentiation tested	> 10
Human White Preadipocytes (HWP) visceral	C-27410 C-27436 C-27438	5,000 cells per cm²	P2	Differentiation tested	> 10

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

Related Products

Product	Size	Catalog Number
Preadipocyte Growth Medium (Ready-to-use)	500 ml	C-27410
Preadipocyte Growth Medium Kit	500 ml	C-27417
Preadipocyte Basal Medium	500 ml	C-27411
Preadipocyte Basal Medium, phenol red-free	500 ml	C-27415
Preadipocyte Growth Medium SupplementMix	for 500 ml	C-39425
Preadipocyte Growth Medium SupplementPack	for 500 ml	C-39427
Preadipocyte Differentiation Medium (Ready-to-use)	500 ml	C-27436
Preadipocyte Differentiation Medium Kit	500 ml	C-27437
Preadipocyte Differentiation Medium SupplementMix	for 500 m	C-39436
Preadipocyte Differentiation Medium SupplementPack	for 500 ml	C-39437
Adipocyte Nutrition Medium (Ready-to-use)	500 ml	C-27438
Adipocyte Nutrition Medium Kit	500 ml	C-27439
Adipocyte Basal Medium	500 ml	C-27431
Adipocyte Basal Medium, phenol red-free	500 ml	C-27435
Adipocyte Nutrition Medium SupplementMix	for 500 ml	C-39438
Adipocyte Nutrition Medium SupplementPack	for 500 ml	C-39439
DetachKit	30 ml 125 ml 250 ml	C-41200 C-41210 C-41220
Cryo-SFM	30 ml 125 ml	C-29910 C-29912
HWP subcutaneous Pellet	1 million cells per pellet	C-14072
HWP visceral Pellet	1 million cells per pellet	C-14073

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 90 93 32 (ligne verte)
Téléfax: 0800 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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