provitro

Provitro AG
Charité Campus Mitte
Charitéplatz 1
10117 Berlin
tel +49.30.450 578 358
fax +49.30.450 578 919
sales@provitro.de
www.provitro.de

Human keratinocytes, dermis (HKER-D), cryo

Cat.-No.: 121 0512 (500,000 cells / cryovial)

Maintenance of HKER-D

Check the cryovial for signs of damage during dispatch. Since the cryopreserved HKER-D arrived on dry ice, they have to be transferred immediately to a deep freezer (-80°C) for a storage time of up to one week or to be thawed according to the procedure stated below. For
In the case of longer storage, transfer the vial directly to liquid nitrogen (-196°C).

Thawing of cryopreserved HKER-D:

1. Transfer the cryovial straight to a water bath with a temperature of 37°C. To achieve a rapid thawing, gently stir the cryovial repeatedly. Few seconds before thawing is completed transfer the vial into a laminar flow hood. Decontaminate the outer surface of the cryovial, and proceed immediately with the following steps under sterile conditions.
2. Within in a laminar flow hood, transfer aseptically the thawed cell suspension into a 15 ml centrifuge tube. Add 10 ml of keratinocyte growth medium, seal the tube with its screw cap, and centrifuge it at 250xg for 5 min.
3. Remove aseptically the supernatant, and re-suspend the cell pellet in 10 ml of provitro's keratinocyte growth medium using a serological pipette. **Check cell number and viability.**
4. Transfer the entire volume of HKER-D suspension into two 25 cm² culture flasks (5 ml each). The advantage of using two 25 cm² flasks instead of one larger flask is that it helps to reduce the risk of loosing all cells. So, if you experience difficulties in passaging the first 25 cm² flask, there is still another flask left for further use. In order to prevent contamination make sure that there are no traces of medium left on the inner/outer part of the flask neck.
5. Place the cell culture flasks in an incubator at 37°C, steam saturated and 5 % (V/V) CO_2. Close the screw lids on each culture flask by half a turn only to allow gas exchanges to take place.
6. Change the keratinocyte growth medium after the first 24 hrs to remove unattached cells, then every 48-72hrs. **Feed the cells only with culture medium that has been warmed up.**
7. The cells are ready for sub-culturing having reached a confluence of 75 %. If the cell layer is allowed to grow too dense they will suffer irreversible contact inhibition leading to ceased proliferation. **For subculturing, use the reagents recommended in the accompanying analysis certificate, only.**
8. Recommended seeding density of HKER-D: **> 6,000 cells per cm²**

Description:

Following provitro's standard operating procedures, the HKER-D cultures are isolated from human tissue, then transferred into a primary cell culture, and finally aliquoted and frozen.

Proliferative capacity of HKER-D:

Provitro's HKER-D cultures are derived from original tissue (in vivo state) using careful methods,. They are not transformed or mutated and have a limited *in-vitro* lifespan. All HKER-D batches are tested by provitro for their proliferative capacity using provitro's culture reagents as outlined in the accompanying certificate of analysis.

Quality control:

All HKER-D cultures from provitro are subject to comprehensive quality tests, summarized in the accompanying certificate of analysis.

Warning note:

Concerning use of biological material:
Provitro's primary cell cultures are of human or animal origin, and no known test procedures can ensure the total absence of infectious agents. All products should therefore be handled following safety precautions as if they were infectious.

In vitro laboratory use only.

Not intended for any human or animal diagnostic or therapeutic use.