Product Information

Human Conjunctival Epithelial Cells (HConEpC)

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>10HU-097</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Homo sapiens</td>
</tr>
<tr>
<td>Cell Number</td>
<td>0.5 million cells/vial</td>
</tr>
<tr>
<td>Storage Temperature</td>
<td>Liquid Nitrogen</td>
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</tbody>
</table>

Description

The human conjunctiva is a translucent membrane that lines the inner surface of the eyelids and covers the sclera. It is overlaid by two layers of stratified, non-keratinized conjunctival epithelial cells (HConEpC). HConEpC can be distinguished from the corneal epithelium by a specific expression profile of keratins [1]. HConEpC is covered by mucin-rich glycocalyx, which promotes tear adherence, prevents pathogen penetrance, and provides lubrication. Decrease or loss of mucin/glycocalyx production generates squamous metaplasia, which may lead to dry eye and ocular surface diseases [2]. HConEpC also respond to histamine by stimulating phosphatidylinositol turnover and secreting cytokines, and as a result may be a good target when developing topical ocular drugs to treat allergic conjunctivitis [3].

iXCells Biotechnologies provides high quality HConEpC, which are isolated from human conjunctiva and cryopreserved at P1, with >0.5 million cells in each vial. HConEpC express cytokeratin-18 and -19 and are characterized by their cobble stone morphology in serum-free culture. They are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi and can further expand for 10 population doublings in Corneal Epithelial Cell Growth Medium (Cat# MD-0064) under the condition suggested by iXCells Biotechnologies.

Figure 1. Human Conjunctival Epithelial Cells (HConEpC) (phase contrast).
Product Details

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Human conjunctiva</th>
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<tbody>
<tr>
<td>Package Size</td>
<td>0.5 x 10^6 cells/vial</td>
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<tr>
<td>Passage Number</td>
<td>P1</td>
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<td>Shipped</td>
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<td>Growth Properties</td>
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<tr>
<td>Media</td>
<td>Corneal Epithelial Cell Growth Medium (Cat# MD-0064)</td>
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</table>

Protocols

Thawing of Frozen Cells

1. Prepare a poly-L-lysine-coated culture vessel (2 μg/cm², T75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 μl of poly-L-lysine stock solution (10 mg/ml). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour).
2. Rinse the poly-L-lysine-coated vessel twice with sterile water and then add 15 ml of Corneal Epithelial Cell Growth Medium (Cat# MD-0064). Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.
3. To thaw the HConEpC, put the vial in 37°C water bath with gentle agitation for ~1 minute. Keep the cap out of water to minimize the risk of contamination.
4. Pipette the cells into a 15 ml conical tube with 5 ml fresh Corneal Epithelial Cell Growth Medium (Cat# MD-0064).
5. Centrifuge at 1,000 rpm (~220 g) for 5 minutes under room temperature.
6. Remove the supernatant and resuspend the cells in fresh Corneal Epithelial Cell Growth Medium (Cat# MD-0064).
7. Culture the cell in the equilibrated, poly-L-lysine-coated culture vessel. A seeding density of 5,000 cells/cm² is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture. It is also important that cells are plated in poly-L-lysine-coated culture vessels to promote cell attachment.
8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells.
9. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
10. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent.

Safety Precaution: it is highly recommended that protective gloves and clothing should be used when handling frozen vials.

**Subculturing**

1. Subculture when the culture reaches 90% confluency or above.
2. Prepare poly-L-lysine-coated culture vessels (2 μg/cm²) one day before subculture.
3. Warm Corneal Epithelial Cell Growth Medium (Cat# MD-0064), trypsin/EDTA solution, and DPBS to room temperature. We do not recommend warming reagents and medium in a 37°C water bath prior to use.
4. Remove the culture medium from the culture vessel. Rinse the cells with DPBS.
5. Add 3ml of 0.25% Trypsin-EDTA to the T75 flask and incubate for ~3 minutes at 37°C. Neutralize the enzyme by adding 2-3 volumes of cell culture medium.
6. Centrifuge at 1,000 rpm (~220 g) for 5 minutes and resuspend the cells in desired volume of medium.
7. Seed the cells in a new poly-L-lysine-coated culture vessel at 5,000 cells/cm².

**References**


**Disclaimers**

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