# 3D-NET (EU FP7 2013 - 612218/3D-NET)

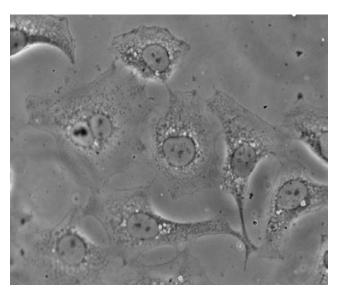
## **3DNET SOPs**





#### **CELL CULTURE OF OCULAR TISSUES-DERIVED CELL LINES**

#### **IOBA Normal Conjunctival Epithelial (IOBA-NHC) Cell Line**



#### Reference:

Diebold Y, Calonge M, Enríquez de Salamanca A, Callejo S, Corrales RM, Sáez V, Siemasko KF, Stern ME. Characterization of a spontaneously immortalized cell line (IOBA-NHC) from normal human conjunctiva. Invest Ophthalmol Vis Sci. 2003; 44:4263–4274.

#### **Characteristics:**

The IOBA-NHC cell line is derived from normal human conjunctival epithelial cells that were spontaneously immortalized.

Positive for diverse cykokeratins, mucins, and e-cadherin.

## **Culture conditions:**

Dulbecco's Modified Essential Medium/F-12 mixture (DMEM/F-12) with GlutaMAXTM-I supplemented with 10% fetal bovine serum (FBS),  $1\mu g/mL$  bovine pancreas insulin, 2ng/mL mouse epidermal growth factor (EGF),  $5\mu g/mL$  hydrocortisone, 50U/mL penicillin,  $50\mu g/mL$  streptomycin, and  $2.5\mu g/mL$  fungizone.

Cells are maintained at 37° C in a 5% CO2 atmosphere. Culture medium needs to be changed every 2-3 days. Usually, 7-8 mL fresh medium is added (25 cm² flask).

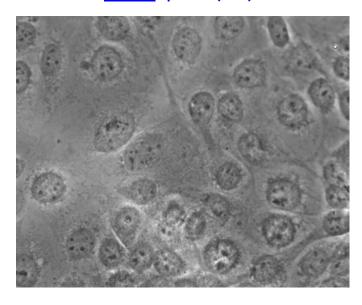
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## **3DNET SOPs**

#### **HOW-TO-DO PRACTICAL GUIDE**



## **Human Corneal Epitelial (HCE) Cell Line**



#### Reference:

Araki-Sasaki, K., Ohashi, Y., Sasabe, T., Hayashi, K., Watanabe, H., Tano, Y., Handa, H. An SV40-immortalized human corneal epithelial cell line and its characterization. Invest Ophthalmol Vis Sci. 1995; 36: 614-621.

#### **Characteristics:**

The HCE cell line is derived from normal human corneal epithelial cells that were immortalized by using SV-40.

Positive for diverse cytokeratines, and e-cadherin.

#### **Culture conditions:**

Dulbecco's Modified Essential Medium/F-12 mixture (DMEM/F-12) with GlutaMAXTM-I supplemented with 15% fetal bovine serum (FBS),  $5\mu g/mL$  recombinant human insulin, 10ng/mL mouse epidermal growth factor (EGF), 62.5U/ml penicillin, and  $62.5\mu g/ml$  streptomycin.

Cells are maintained at 37º C in a 5% CO2 atmosphere. Culture medium needs to be changed every 2-3 days. Usually, 7-8 mL fresh medium is added (25 cm² flask).

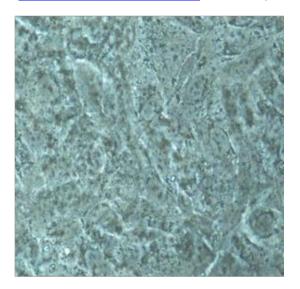
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## **Human Retinal Pigment Epithelium Cell Line (ARPE-19)**



#### Reference:

American Type Culture Collection (ATCC® CRL-2302™)

#### **Characteristics:**

The ARPE-19 cell line is derived from normal human retinal pigment cells that were spontaneously immortalized.

Positive for CRALBP and RPE65.

#### **Culture conditions:**

Dulbecco's Modified Essential Medium/F-12 mixture (DMEM/F-12) supplemented with 10% fetal bovine serum (FBS), 100U/mL penicillin, and 0.1 mg/mL streptomycin.

Cells are maintained at 37º C in a 5% CO2 atmosphere. Culture medium needs to be changed every 2-3 days. Usually, 7-8 mL fresh medium is added (25 cm² flask).