**IMMEDIATELY UPON RECEIPT:**

1. Don protective gloves, clothing, and face shield before handling frozen vials, dry ice or liquid nitrogen.
2. Check shipment container for breakage or leakage.
3. Transfer cryovial from the dry ice packaging to liquid nitrogen storage until ready for use.

**Storage Temperature:** Liquid Nitrogen Vapor Phase (-196°C)

**Biosafety Level:** 1

**Product Use**

This product is for research use only. It is not approved for use in humans, animals, or in vitro diagnostic procedures.

**Product Description**

Primary adult human Retinal Pigment Epithelial cells derived from cadaveric donor retina.

- All donors are selected for optimal tissue viability conditions in the absence of known retinal disease.
- Refer to Page 2 for donor and cell line-specific data.

**Preparing the Medium**

The base medium for ahRPE culturing is a DMEM:F12 and αMEM solution containing 2% (v/v) Fetal Bovine Serum (FBS) plus additional supplements (Fernandes et al., 2018).

- For initial cell thawing and plating, supplement the base medium by adding enough FBS to achieve a final concentration of 10% (v/v) FBS.

**Preparing Culture Surfaces**

For optimal cell attachment, use vessels coated with Corning® Synthemax™ II-SC Substrate.

1. Prepare a 25 μg/ml Synthemax™ II-SC Substrate solution according to manufacturer protocol.
2. Coat vessel surfaces by adding the recommended coating volume listed in the manufacturer protocol.
3. Incubate coated vessel at room temperature for ≥ 2 hours.
4. Remove coating solution and allow culture surfaces to dry before use.

**Thawing the Cells**

1. Warm 25 ml of medium, supplemented to 10% (v/v) FBS, to 37°C.
2. Dispense 8 ml of medium into a sterile 15 ml centrifuge tube.
3. Thaw the cryovial in a 37°C water bath for 3 minutes and decontaminate with 70% ethanol.
4. Transfer the thawed cell suspension to the centrifuge tube containing the 8 ml of medium.

5. Rinse the interior of the cryovial with an additional 1 ml of medium and transfer to the centrifuge tube.
6. Centrifuge the cell suspension at 259 × g for 5 minutes; discard the supernatant.

**Plating the Cells**

1. Re-suspend the pelleted cells in medium supplemented to 10% (v/v) FBS at a concentration of ~1.0 × 10^5 cells/ml. Each cryovial contains ≥ 5.0 x 10^5 cells.
2. Plate the cells at a density of ~1.0 x 10^5 cells/1.9 cm² surface area.
3. Allow the vessel to rest undisturbed for 10 minutes to achieve even cell distribution.
4. Incubate the cells at 37°C, 5% CO₂.

**Replacing the Medium**

Every 4th day, remove ⅔ of the vessel’s medium and immediately replace with an equal volume of fresh base medium warmed to 37°C.

- For most applications, the cells should be cultured for a minimum of 4 weeks.

**References**

**Donor Information**

- **Age:** 57
- **Gender:** Female
- **Race:** Caucasian

**Date & Time of Death:** 2019-12-15 09:40
**Date & Time of Preservation:** 2019-12-15 18:49

**Cause of Death**
- Cranial Hemorrhage (Non-Traumatic)
- Hemorrhagic Shock, Hypertension

**Medical History**
- Chronic Cortical Hemorrhage, Hemorrhagic Stroke,
- Cerebrovascular Accident (2018), Hypertension, Altered Mental Status, Loss of Brainstem Function, Anxiety, Hysterectomy,
- Cigarette Smoker, Alcohol Abuse, Asthma

**Ocular History**
- Cataracts Surgery (OU)

**Blood Sample Type:** Post-Mortem
**Serology Lab:** VRL Northeast

**Hep B Core Total Ab** 2019-12-17 NON-REACTIVE
**Hep B Surface Ag** 2019-12-17 NON-REACTIVE
**Hep C Ab** 2019-12-17 NON-REACTIVE
**HIV I/II Ab** 2019-12-17 NON-REACTIVE
**RPR** 2019-12-17 NON-REACTIVE
**HBV-NAT** 2019-12-17 NON-REACTIVE
**HCV-NAT** 2019-12-17 NON-REACTIVE
**HIV-NAT** 2019-12-17 NON-REACTIVE
**HTLV I/II Ab** 2019-12-17 NON-REACTIVE

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**InvivoGen® PlasmoTest™ Mycoplasma Detection Results**

2020-06-10 NOT DETECTED

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**Researcher:**
**Shipping Method:**
**Packaging Technician:**
**Date & Time of Shipment:**