NEUROMICS

Human Retinal Pigment Epithelial Cells

Catalog Number	RPE001
Storage	Liquid Nitrogen
Cell Number:	Frozen Vial (> 5 x 105cells/vial)
Viablitliy	≥80% when thawed

Caution: Proper precautions must be taken to avoid exposure. Always wear proper protective equipment (Gloves, safety glasses, etc.) when handling these materials. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination. The listed dilutions are for recommendation only and the ender users should optimize the final conditions.

General Information

HRPEs are initiated by dissecting Retinal Pigment tissue and digestion with collagenase. HRPEs are separated/purified and offered in frozen vial format (the cells are provided @ passage 3). HRPEs growth medium (contains 10% serum and growth supplements, Alpha-33) is recommended for cell culture and these cells have a minimum average population doubling capacity > 8 when cultured following the detailed protocol described below).

Characterization of the cells:

HRPEs are positive for REPs-specific markers CRALBP and RPE-65 HRPEs are negative for HIV-1, HBV, HCV, and mycoplasma.

Product Use: For research use only.
Shipping: Shipping on dry ice or in LN2 is required.

Handling of Arriving Cells

When you receive the cells in a frozen vial, you can transfer the vial of cells into a -80ºC freezer for short period storage or a liquid nitrogen tank for long term storage. Thaw the cells in a 37°C water bath, and then transfer the cells into a T25 flask pre-coated with Alphabiocoat as described in details in Subculture Protocol.

Subculture Protocol

1) Pre-coating of T25 flasks: Add 2ml of Alphabiocoat into one T25 flask and make sure whole surface of the flask is covered with the coating solution. Five minutes later, dispose excessive coating solution by aspiration and the flask is ready to be used (no need for overnight incubation when using our coating solution). Other extracellular matrix can be used including gelatin, collagen, and fibronectin and you are advised to test the conditions for using those materials in advance.

2) Rinse the cells in T25 flask with 5ml HBSS (**Room Temperature, RT**) twice.

3) Add 2ml of Trypsin/EDTA (**RT**) into one T25 flask (make sure the whole surface of the T25 flask is covered with Trypsin/EDTA), and gently dispose the excessive Trypsin/EDTA solution **within 60 seconds** with aspiration.

www.neuromics.com

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
Phone 866-350-1500 • fax 612-677-3976 • email: pshuster@neuromics.com

4) Leave the T25 flask with the cells at 37C for extra 1-2 minute (the cells usually will detach from the surface within 1-2 minutes). You can monitor the cells under microscope and when most of cells become rounded up, hit the flask against the bench surface, and the cells will move on the surface of the flask when monitoring under microscope.

5) Add 5ml Trypsin Neutralization Buffer and spin the cells down with 800g for 5 minutes.

6) Re-suspend the cell pellet with 10- 15ml of HRPEs Growth Medium and the cell suspension is transferred directly into 2 or 3 pre-coated T25 flasks (5ml each, and the cells are sub-cultured at 1 : 2 to 1 : 3 ratios)

7) Change medium every 2-3 days and cells usually become confluent within 7 days (when split at a 1:3 ratio).

Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439
Phone 866-350-1500 • fax 612-677-3976 • email: pshuster@neuromics.com