Piezo1 knock-out HEK293T

CRL-3519™

Description

The Piezo1 knock-out HEK293T cell line can be used to assess ion channel activity by electrophysiology without any interference from endogenous mechanically activated currents caused by the ion channel Piezo1.

Organism: *Homo sapiens*, human
Tissue: kidney
Morphology: epithelial
Growth properties: Adherent
Cells per vial: Approximately 2.0 to 3.0 x 10^6
Volume: 1.0 mL

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories*

(BMBL), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Complete medium: The base medium for this cell line is Dulbecco's Modified Eagle's Medium (DMEM) (ATCC 30-2002). To make the complete growth medium, add the

following components to the base medium: 10% Fetal Bovine Serum (heat inactivated) (ATCC 30-2020), 2mM L-glutamine (ATCC 30-2214)

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. ;Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Rinse cells with 10 mL PBS . Note: do not remove PBS. The PBS should be transferred to the centrifugation tube to collect the detached cells while rinsing. The tube with PBS washed cells and the tube with neutralized cells can be combined and centrifuged. Add 2.0 to 3.0 mL of 0.25% (w/v) Trypsin- 0.53

mM solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

3. Remove dissociation agent by centrifugation. 150 to 400 x g; 8 to 12 minutes. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 1.0 x 10^4 and 4.0 x 10^4 viable cells/cm^2.
5. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 1.0 X 10^4 and 3.0 X 10^4 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: Subcultivation every 2-3 days
Culture maintenance:

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x *g* for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Reagents for cryopreservation: DMEM + 10% Heat-Inactivated FBS + 2 mM L-Glutamine + 5% DMSO

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Piezo1 knock-out HEK293T (ATCC CRL-3519)

References

References and other information relating to this material are available at www.atcc.org.

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