PERSONATHER
PERSONALIZED THERAPY

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PRODUCT DATA SHEET

PRODUCT: Research Tool

CATALOG NUMBER: RT 02001-PER

DESIGNATION: DK-MG^{lowexoKRASG12V}

ORGANISM: Homo sapiens, human

QUANTITY & CONCENTRATION: freeze in medium with 10% of DMSO, 1 mL, 1x10^6 cells/vial

CELLULAR ORIGIN: glioblastoma

CHARACTERISTIC: adherent cell culture

MORPHOLOGY: epithelial cells

SHIPPED IN: dry ice

BACKGROUND/DESCRIPTION:

The RT 02001-PER cell line was created from a stable cell line that was transduced with a plasmid encoding the KRAS G12V sequence. The expression of KRAS G12V was confirmed by Western Blotting. The CLTH EGFR/EGFRvIII cell line also has puromycin resistance gene.

QUALITY CONTROL SPECIFICATION: mycoplasma contamination – not detected.

HANDLING INFORMATION

UNPACKING & STORAGE INSTRUCTIONS:

1. Check the container for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

COMPLETE MEDIUM:

The base medium for this cell line is RPMI 1640. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (FBS) to a final concentration of 10%.

TEMPERATURE: 37°C

ATMOSPHERE: 95% Air, 5% CO_2

HANDLING PROCEDURE:

To insure 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.

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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 growth medium and spin at approximately 200 x g for 3 to 5 minutes.
4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO2 in air atmosphere is recommended if using the medium described on this product.

SUBCULTURING PROCEDURE:

Volumes are given for a 25 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with phosphate buffer saline (PBS) w/o magnesium and calcium ions to remove all traces of serum which contains trypsin inhibitor.
3. Add 0.5 to 1 mL of 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 4.0 to 5.0 mL of complete growth medium and aspirate cells by gently pipetting and transfer to a centrifuge tube.
5. Spin at approximately 200 x g for 3 to 5 minutes.
6. Remove supernatant and resuspend cell pellet with the recommended complete growth medium and dispense into a 25 cm^2 or a 75 cm^2 culture flask.
7. Incubate cultures at 37°C.

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT. Subculture at least twice per week at 80% confluence or less.

Subcultivation Ratio: Inoculate 3 to 5 X 10^3 cells/cm^2

Medium Renewal: Twice per week

Reagents for cryopreservation: complete growth medium with 10% DMSO

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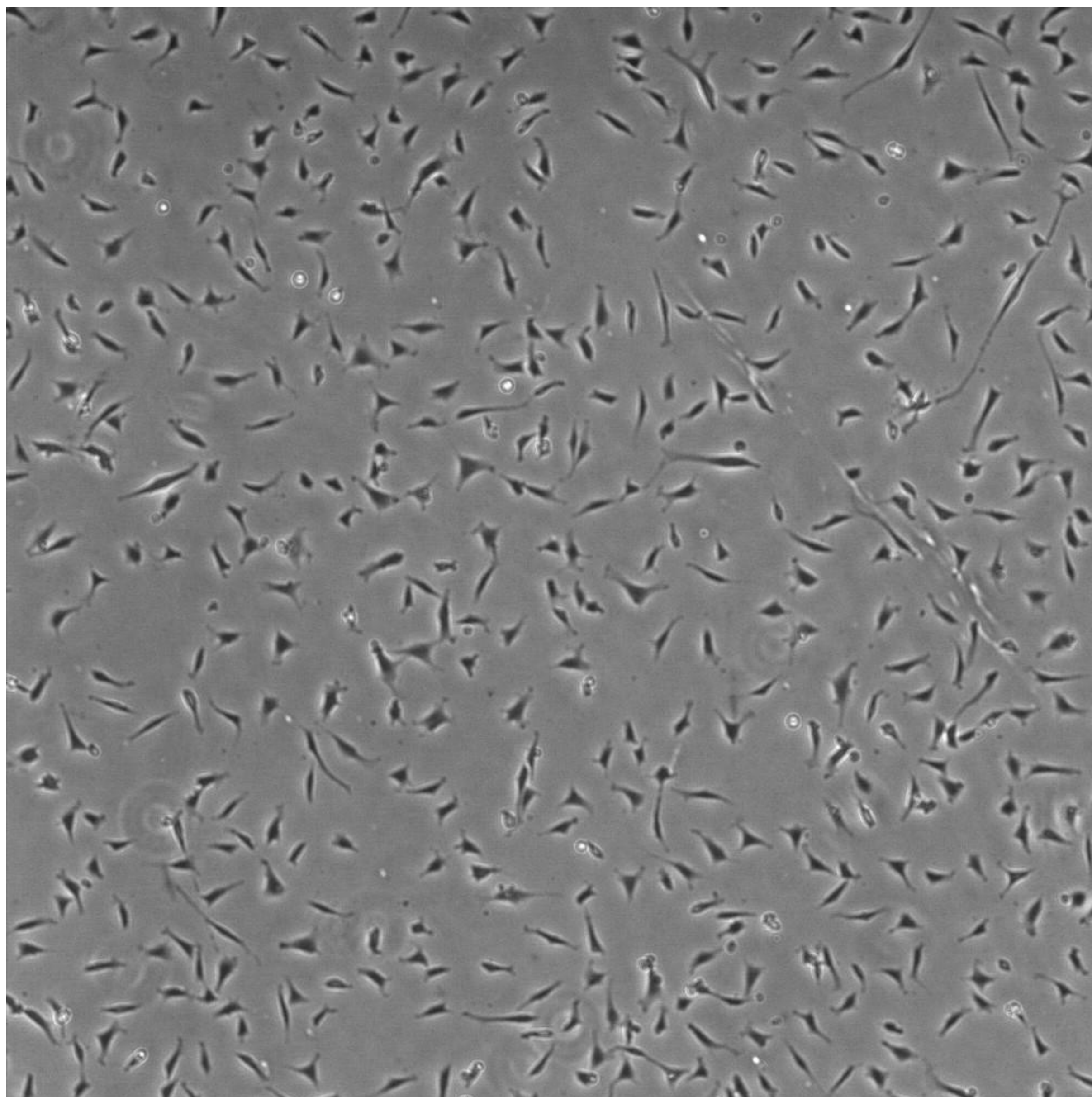
IMAGES:



SAFETY PRECAUTION:

Personather Ltd. highly recommends using protective gloves and clothing. It is also important to avoid breathing vapor, avoid skin contact or swallowing.

WASTE DISPOSAL:

Chemical wastes should always be returned to special company responsible for utilizing such type of wastes.

PERSONATHER LTD. WARRANTY

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The viability of Personather products is warranted for 30 days from the date of shipment and is valid only if the product is stored according to the information included in this product information sheet. Since this product is under development – we cannot guarantee same functionality in all experimental conditions.

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REFERENCES:

Wlodarczyk A, et al. Phenotypical Flexibility of the EGFRvIII-Positive Glioblastoma Cell Line and the Multidirectional Influence of TGFβ and EGF on These Cells-EGFRvIII Appears as a Weak Oncogene. Int J Mol Sci. 2022 Oct 12;23(20):12129. doi: 10.3390/ijms232012129.

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