MAPK7 (p.D200A) Point Mutation in Ishikawa Cell Line

User Guide

- Basic Information

Item	Details
Catalog Number	EDC07573
Product Name	MAPK7 (p.D200A) Point Mutation in Ishikawa Cell Line
Cell Morphology	Adherent
Digestion Time	1 ~2min
Passage Ratio	1:3~1:4
Target Gene	MAPK7
Gene ID	5598
Cell Density	≥1 × 10^6 cells/mL
Complete Culture Medium	DMEM+10%FBS
Freezing Medium	55% basic culture medium+40% FBS+5% DMSO
Remarks	

- Editing Results

Editing Results	Sequencing Data
MAPK7, p.D200A, c.599A>C, Homozygous	The corresponding sequencing validation data for this edited cell line is provided separately in the attachment.

- **Cell Receipt Instructions**

1. **Frozen Cells**

If the cells are shipped on dry ice, please transfer them immediately to liquid nitrogen for storage upon receipt, or proceed directly with cell thawing.

2. **Live Cells**

Upon receiving live cells, disinfect the exterior of the T25 culture flask with 75% ethanol. Then, place the flask in a 37°C, 5% CO_2 incubator for 2 hours. After incubation, examine the cells under a microscope to assess attachment and confluency. Capture images of two different fields of view at both 100× and 40× magnification for record-keeping.

If the cell confluency reaches 80% or higher, proceed with passaging. If confluency is below 80%, discard the old culture medium, replace with fresh complete medium, and continue culture.

Note: Culture flasks filled with old medium are not suitable for immediate passaging.

- **Cell Thawing and Recovery Instructions**

1. Preheat a water bath to 37°C.
2. Prewarm the complete culture medium suitable for the specific cell line to 37°C.
3. Prepare a 15 mL centrifuge tube containing 6 mL of prewarmed complete medium.
4. Retrieve the frozen cells from liquid nitrogen and gently swirl the cryovial in the 37°C water bath. Thaw the cells quickly within approximately 2 minutes, leaving only a small ice core.

Note: Do not immerse the vial cap in water, and do not seal the vial with parafilm.

5. Transfer the vial to a biosafety cabinet. Wipe the exterior with 75% ethanol before opening.
6. Using a pipette, transfer the cell suspension into the prewarmed complete medium in the centrifuge tube.
7. Centrifuge at 500 × g for 5 minutes.
8. After centrifugation, check that the supernatant is clear and that the cell pellet is intact. Carefully aspirate the supernatant under sterile conditions, add 1 mL of complete medium, and gently resuspend the cell pellet.
9. Seed the cells into one T25 flask or a culture vessel with equivalent growth area, adding 4 mL of complete medium per flask.

10. Gently mix the cells and incubate in a 37°C, 5% CO_2 incubator (culture conditions depend on the cell line and medium).
11. On the following day, assess cell status:

Adherent cells: If well attached, replace with fresh complete medium. If round but not attached, continue culture for 24 hours before medium change. Subsequently, replace medium every 2-3 days based on growth. Passage cells when confluency reaches ≥80%. If growth is slow or confluency is low, medium change frequency can be reduced.

Suspension cells: Thaw in a relatively small culture vessel using complete medium containing 20% serum. If cells are healthy, replace with fresh complete medium. If cells appear gray or unhealthy, continue culture for 24 hours and re-assess. If live cells are observed, medium can be refreshed. If cell status does not improve, contact our technical support.

- **Cell Passage**

Adherent Cells

1. Prewarm the complete culture medium, PBS, and trypsin to 37°C.
2. Aspirate the spent medium from the culture vessel.
3. Gently add PBS to rinse the cells once (approximately 2 mL for a T25 flask). Handle gently and ensure thorough washing without disturbing the cell layer. Gently rock the vessel back and forth several times, then aspirate the PBS. This step removes residual serum and divalent ions (Ca^{2+} and Mg^{2+}) that may inhibit trypsin activity.
4. Add trypsin (approximately 1 mL for a T25 flask) and gently shake to ensure even coverage of the cell surface. Place the flask in the incubator for digestion.
5. Observe the digestion under a microscope. When approximately 70–80% of cells have rounded up and detached, gently tap the side of the flask to facilitate detachment.
6. Immediately add 2–3 times the trypsin volume of complete medium (approximately 3 mL for a T25 flask) to neutralize the trypsin. Gently swirl to mix.
7. Use a pipette to gently flush the bottom surface to detach remaining cells. Avoid vigorous pipetting or bubble formation to prevent cell damage and loss.
8. Collect the entire cell suspension and centrifuge at 500 × g for 5 minutes.

9. Carefully aspirate the supernatant, add 1 mL of complete medium, and gently resuspend the cell pellet until homogeneous.
10. Reseed the cells at an appropriate split ratio. A 1:2 split is recommended for the first passage. If cells reach confluency within two days, increase the split ratio; if confluency is not reached after three to four days, decrease it accordingly. Adjust based on actual growth performance.
11. Gently mix the cells and place them in a 37°C, 5% CO_2 incubator. For non-vented culture flasks, loosen the cap slightly to allow adequate gas exchange before incubation.
12. Observe cell morphology the next day. If a significant number of dead cells are observed, replace with fresh medium. Continue to monitor daily and passage or cryopreserve the cells when confluency exceeds 80%.

Suspension Cells

1. Prewarm the complete culture medium, PBS, and trypsin to 37°C.
2. Using a pipette, gently flush the bottom of the culture vessel several times to detach and resuspend the cells evenly.

Note: Avoid vigorous pipetting or bubble formation to prevent cell damage and loss.

3. Collect the entire cell suspension and centrifuge at 500 × g for 4 minutes.
4. Carefully remove the supernatant, then add 1 mL of complete medium under sterile conditions. Gently resuspend the cell pellet until fully dispersed and homogeneous.
5. Reseed the cells at an appropriate split ratio. A 1:2 ratio is recommended for the first passage. If the cells reach high density within two days, the split ratio can be increased; if growth remains slow after three to four days, reduce the ratio accordingly. Adjust flexibly based on cell growth performance.
6. Gently mix the cells and place them in a 37°C, 5% CO_2 incubator. For non-vented flasks, slightly loosen the cap before incubation to ensure adequate gas exchange.
7. Observe the culture the following day. If a large number of dead cells are observed, replace with fresh complete medium. Continue to monitor daily and refresh the medium as needed. Once the culture reaches optimal density (comparable to ~80% confluency for adherent cells), proceed with the next passage or cryopreservation.

- **Cell Cryopreservation**

1. Collect the cell pellet following standard passaging procedures and resuspend cells in an appropriate volume of complete medium according to pellet size.
2. Gently pipette to mix thoroughly and take a 20 µL aliquot for cell counting.
3. Centrifuge the cell suspension at 500 × g for 5 minutes at room temperature. After centrifugation, carefully aspirate the supernatant and resuspend the pellet in 1-2 mL of prechilled (4°C) freezing medium.
4. Adjust the cell concentration to $1 \times 10^6 – 1 \times 10^7$ cells/mL using the freezing medium.
5. Aliquot 1 mL per cryovial, cap tightly, and label each vial clearly with cell name, passage number, cell count, and freeze date.
6. Place vials into a prechilled (4°C) controlled-rate freezing container (programmed cooling box). Start the freezing program, and within 15 minutes after the program completes, transfer the freezing container to an ultra-low temperature freezer (e.g., -80°C).
7. After overnight equilibration, transfer cryovials to liquid nitrogen storage for long-term preservation.

Precautions

- On receipt of cells shipped at ambient temperature, photograph the shipment immediately and record any leakage or container damage.
- Wipe the exterior of culture vessels with 75% ethanol and inspect cell status under a microscope. Allow the culture to rest in the incubator for 2-4 hours to stabilize before further manipulation.
- Read the cell datasheet carefully prior to cryopreservation to confirm attachment properties, recommended basal medium, split ratio, and medium-change frequency.
- Perform all cryopreservation steps under sterile conditions using prechilled freezing medium and consumables.
- Maintain photographic records of cell morphology and growth for quality tracking.
- If any abnormalities are observed or if you have questions regarding thawing, passaging, or cryopreservation procedures, contact our technical support promptly.

Advantages

Diverse Species

Over 100 wild-type cell lines are available, covering multiple species including human, mouse, chicken, pig, and cattle, with applications across a wide range of research fields.

STR Authenticity Verification

Each cell line undergoes STR or species identification and rigorous quality control to ensure accurate origin and identity.

Experimental Validation

All cell lines have been experimentally validated through gene-editing assays, making them suitable for most gene-editing and molecular biology studies.

Authoritative Sources

All cell lines are sourced from reputable repositories such as ATCC and the Chinese Academy of Sciences, ensuring low passage numbers, high viability, and stable cell conditions.