MDA-T32-Cas9 Cell Line User Guide

▶ Product Information

Cas9 protein is the core functional component of the CRISPR gene editing system. Its unique molecular mechanism and efficient DNA cleavage ability make it the cornerstone of modern gene editing technologies. The Cas9 stable cell line is a cell model that allows for the continuous and stable expression of Cas9 protein, maintaining cell viability and stability, and providing an efficient tool for gene editing research.

EDITGENE has successfully developed a next-generation Cas9 stable cell line by innovatively integrating the OE-Booste cis-regulatory elements. This new cell line exhibits significant Cas9 expression levels and gene editing efficiency, supporting gene function studies, high-throughput screening, disease model development, and cell therapy research.

Product Name	MDA-T32-Cas9
Catalog No.	EDC01169
Cell Morphology	Adherent
Trypsinization Time	~1 min
Fluorescence	-
Resistance	BSD
Drug Administration	blast=4 μg/mL
Passage Ratio	1:3
Complete Culture Medium	1640+10% FBS+1% GlutaMAX+1% NEAA
Cryopreservation Culture Medium	70% complete culture medium+20% FBS+10% DMSO

▶ Advantages of Cas9 Cell Line:

Excellent Stability

Equipped with the OE-Booster regulatory element, the mRNA stability is optimized, ensuring

continuous and stable expression of the Cas9 protein.

Ease of Use

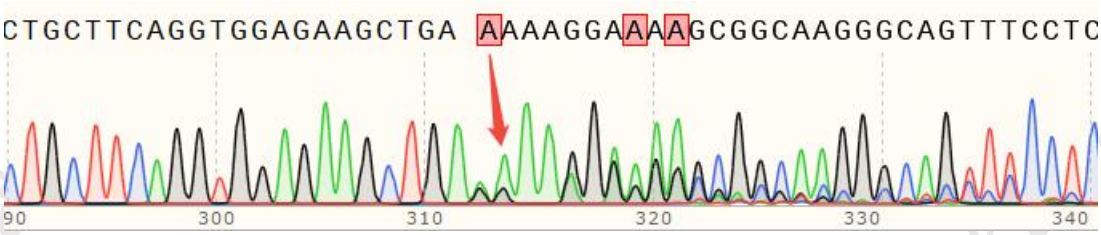
The stable cell line continuously expresses the Cas9 protein, eliminating the need for separate transfection of the Cas9 gene, thus simplifying the workflow.

Broad Applicability

Suitable for gene function studies, library screening, disease model development, and cell therapy research, meeting diverse research needs.

▶ Product Validation Data

Cleavage Verification



Note: The figure above shows sequencing chromatograms from pooled MDA-T32-Cas9 stable cells infected with a lentiviral sgRNA plasmid targeting the OLFML2A gene. Cells were selected with antibiotics for 48 hours before analysis. The red arrows indicate overlapping peaks at the target site, reflecting successful Cas9-mediated cleavage and subsequent genotype alterations—demonstrating effective expression and activity of the Cas9 nuclease, with a cleavage efficiency of 73%.

▶ Cell Receipt

a. Frozen Cells

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

b. Live Cells

Upon receipt, disinfect the external surface of the T25 flask with 75% ethanol. Place the flask in a 5% CO_2, 37°C incubator for 2 hours. After incubation, observe the cell attachment and confluency under a microscope. Take two photos at different fields of view at 100x and 40x magnification. If the

confluency is greater than 80%, proceed with passaging. If the confluency is less than 80%, discard the medium in the flask, replace it with fresh complete medium, and continue incubation.

Note: Do not overfill the culture flask with medium, as it may affect cell culture conditions.

▶ Cell Thawing Procedure

1. Preheat the water bath to 37°C.
2. Prewarm the complete medium suitable for this cell line to 37°C.
3. Prepare a 15 mL centrifuge tube with 6 mL of complete medium.
4. Retrieve the frozen cells from liquid nitrogen and gently swirl the cryovial in a 37°C water bath until only a small ice core remains. Thaw the cells within 2 minutes.

Note: Do not submerge the vial cap in water or seal the cap with parafilm.

5. Transfer the cryovial to a sterile environment. Disinfect the outside of the cryovial with 75% ethanol before opening.
6. Using a pipette, transfer the cell suspension into the prewarmed complete medium in the centrifuge tube.
7. Centrifuge the cell suspension at 500 g for 4 minutes.
8. After centrifugation, check the supernatant for clarity and verify the presence of a pellet. Carefully aspirate the supernatant under sterile conditions, and gently resuspend the pellet in 1 mL of complete medium. Ensure thorough mixing by gentle pipetting.
9. Seed the cells into a T25 culture flask or an equivalent surface area container, adding 4 mL of complete medium.
10. Gently swirl the flask to ensure even cell distribution and incubate at 37°C, 5% CO_2 (the specific environment depends on the cell type and culture medium used).
11. Observe cell status the next day.

- For adherent cells: If cells are well attached, replace with fresh medium. If cells appear rounded but unattached, allow another 24 hours before changing the medium. Subsequently, change the medium every 2-3 days based on cell growth and passage when 80% confluency is reached.
- For suspension cells: If cell viability is good, replace with fresh medium. If cells appear unhealthy or gray, observe for an additional 24 hours before proceeding with medium changes.

▶ Cell Passaging

a. Adherent Cells

1. Prewarm complete medium, PBS, and trypsin to 37°C.
2. Aspirate the supernatant from the culture vessel.
3. Gently add PBS to one side of the vessel (approximately 2 mL for a T25 flask) to wash the cells. Ensure gentle washing and thorough coverage without disturbing the cell layer. Aspirate the PBS by tilting the flask back and forth.
4. Add 1 mL of trypsin to the T25 flask and distribute evenly. Place the flask in an incubator for digestion.
5. Observe under a microscope for cell detachment (70%-80% cells should round up). Gently tap the flask to detach cells from the surface.
6. Immediately add 2-3 times the volume of complete medium to neutralize the trypsin and gently mix to stop digestion.
7. Collect the cell suspension and pipette the bottom of the flask to ensure all cells are detached. Avoid creating bubbles that could damage cells.
8. Centrifuge the collected cell suspension at 500 g for 5 minutes.
9. After centrifugation, aspirate the supernatant, resuspend the pellet in 1 mL of complete medium, and gently mix.
10. Plate the cells at the appropriate ratio. For the first passage, we recommend a 1:2 ratio. If the cells reach confluency within two days, you can increase the ratio. If it takes 3-4 days to reach confluency, reduce the passaging ratio accordingly.
11. Gently swirl the flask and incubate at 37°C, 5% CO_2 (for vented flasks, loosen the cap before placing in the incubator to allow proper gas exchange).
12. Observe cell status the next day. If a large number of dead cells are present, change the medium. Afterward, change the medium daily based on cell growth until they reach 80% confluency, at which point passaging or cryopreservation is necessary.

b. Suspension Cells

1. Prewarm complete medium, PBS, and trypsin to 37°C.
2. Collect the cell suspension and pipette the bottom of the flask to ensure all cells are detached. Avoid creating bubbles that could damage cells.
3. Centrifuge the collected cell suspension at 500 g for 4 minutes.
4. After centrifugation, aspirate the supernatant, resuspend the pellet in 1 mL of complete medium, and gently mix.

5. Plate the cells at the appropriate ratio. For the first passage, we recommend a 1:2 ratio. If the cells reach confluency within two days, you can increase the ratio. If it takes 3-4 days to reach confluency, reduce the passaging ratio accordingly.

6. Gently swirl the flask and incubate at 37°C, 5% CO_2 (for vented flasks, loosen the cap before placing in the incubator to allow proper gas exchange).

7. Observe cell status the next day. If a large number of dead cells are present, change the medium. Afterward, change the medium daily based on cell growth until they reach 80% confluency, at which point passaging or cryopreservation is necessary.

c. Semi-Adherent and Semi-Suspension.

1. Prewarm complete medium, PBS, and trypsin to 37°C.

2. Collect the cell suspension and pipette the bottom of the flask to ensure all cells are detached. Avoid creating bubbles that could damage cells.

3. Gently add PBS to one side of the vessel (approximately 2 mL for a T25 flask) to wash the cells. Ensure gentle washing and thorough coverage without disturbing the cell layer. Aspirate the PBS by tilting the flask back and forth.

4. Add 1 mL of trypsin to the T25 flask and distribute evenly. Place the flask in an incubator for digestion.

5. Observe under a microscope for cell detachment (70%-80% cells should round up). Gently tap the flask to detach cells from the surface.

6. Immediately add 2-3 times the volume of complete medium to neutralize the trypsin and gently mix to stop digestion.

7. Collect the cell suspension and pipette the bottom of the flask to ensure all cells are detached. Avoid creating bubbles that could damage cells.

8. Centrifuge the collected cell suspension at 500 g for 5 minutes.

9. After centrifugation, aspirate the supernatant, resuspend the pellet in 1 mL of complete medium, and gently mix.

10. Plate the cells at the appropriate ratio. For the first passage, we recommend a 1:2 ratio. If the cells reach confluency within two days, you can increase the ratio. If it takes 3-4 days to reach confluency, reduce the passaging ratio accordingly.

11. Gently swirl the flask and incubate at 37°C, 5% CO_2 (for vented flasks, loosen the cap before placing in the incubator to allow proper gas exchange).

12. Observe cell status the next day. If a large number of dead cells are present, change the medium. Afterward, change the medium daily based on cell growth until they reach 80% confluency, at which point passaging or cryopreservation is necessary.

Note: To maintain the stable expression of Luciferase gene, culture with drug administration is recommended.

▶ Cell Cryopreservation

1. Collect cells as per the passaging protocol and adjust the volume of medium based on the size of the pellet.
2. Gently resuspend the pellet in cold cryopreservation medium.
3. Take 20 μL of the suspension for cell counting.
4. Centrifuge at 500 g for 5 minutes at room temperature.
5. Aspirate the supernatant, then resuspend the pellet in 1-2 mL of pre-cooled cryopreservation medium to achieve a density of $1x10^6$ cells/mL.
6. Aliquot the cell suspension into cryovials (1 mL per vial) and label each vial with the cell name, passage number, cell count, and cryopreservation date.
7. Place the cryovials in a pre-cooled freezing container at 4°C and transfer them to an ultra-low temperature freezer within 15 minutes.
8. After overnight freezing, transfer the cryovials to liquid nitrogen storage for long-term preservation.

Precautions

- Upon receiving room temperature cells, inspect the vial for any leakage or damage, and document accordingly.
- Disinfect the culture flask with 75% ethanol and observe the cell condition under a microscope. Place the flask in a cell culture incubator to stabilize for 2-4 hours before handling.
- Carefully review the user manual to familiarize yourself with the cell line, including adherence properties, morphology, base medium, passaging ratios, and frequency of medium changes.
- After stabilization, remove the flask and inspect the cells again. Photograph and record the cell condition, as this will be used for follow-up services. We recommend photographing the cells during

each passage to document growth status.

• If you notice any abnormalities or have questions about the cells, please contact our support team for assistance.

Advantages

Species Diversity

Over 100 types of wild-type cells from species including human, mouse, chicken, pig, and cow, covering a wide range of research areas.

STR Authentication

Each cell line undergoes STR/species authentication and stringent quality control to ensure cell identity.

Experimental Validation

All cell lines in this collection have been validated for gene-editing experiments, making them suitable for most gene-editing applications.

Authoritative Source

All cell lines are sourced from reputable cell banks such as ATCC and the Chinese Academy of Sciences, ensuring low passage, high viability, and optimal cell status.