Data Sheet

MEF SQSTM1^{G427R} Cell Line

SCC467

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Lobar Degeneration (FTLD) are complex major neurodegenerative diseases that are not fully understood. ALS is characterized by loss of motor neurons in the brain and spinal cord while FTLD is typically characterized by degeneration of neurons in the frontal and anterior temporal lobes. Further understanding of both disorders has led them to be categorized under part of the same disease spectrum. They also share common features such as protein and protein-RNA aggregation in motor neurons. This ultimately implicates a problem in key autophagy pathways.[1]

Recent data suggests that TBK1 and SQSTM1 mutations linked with ALS and FTLD disrupt selective autophagy, resulting in neurotoxicity.[1] SQSTM1 is an autophagy receptor protein that mediates the selective autophagy of damaged cell components or proteins that frequently aggregate. The commonality of SQSTM1-associated neuronal inclusions in ALS-FTLD patients appears to indicate its importance as a cellular stress response in which SQSTM1 protein is overproduced to try and clear protein aggregates. It also functions in other signaling pathways such as oxidative stress which has emerged as a contributing factor in ALS.[1] SQSTM1 helps regulate oxidative stress through the KEAP1-NFE2L2 pathway in which SQSTM1 binds with KEAP1 resulting in a downstream antioxidant response. Interestingly, a G427R mutation appears to significantly disrupt this pathway, resulting in a highly impaired NFE2L2 response.

The MEF (Mouse Embryonic Fibroblast) SQSTM1^{G427R} cell line contains a FLAG-tagged SQSTM1 protein in addition to the G427R mutation which affects the Ubiquitin Binding Associated (UBA) domain of the SQSMT1 protein. This cell line can be utilized to continue studying the underlying mechanisms of selective autophagy, ALS-FTLD disease pathogenesis, and oxidative stress mechanisms. Furthermore, understanding of SQSTM1 and ALS-FTLD related mutations remains highly important as neurodegenerative diseases continue to increase in prevalence.

Source

Cell line was derived from immortalized p62/SQSTM1-deficient mouse embryonic fibroblasts (MEFs) which were established by infecting MEFs with a recombinant retrovirus carrying a temperature-sensitive simian virus 40 large T antigen. Flag-SQSTM1-WT was also cloned into the HindIII and XhoI sites of the LPC retroviral vector before undergoing site-directed mutagenesis to obtain the SQSTM1^{G427R} genotype.

Short Tandem Repeat

M18-3: 16	M4-2: 20.3	M6-7: 17,18	M19-2: 13	M1-2: 19	M7-1: 26, 26.2
M1-1: 17	M3-2: 14	M8-1: 16	M2-1: 16	M6-4: 18	M15-3: 20.3, 22.3
M11-2: 16	M17-2: 15	M12-1: 17	M5-5: 17	M13-1: 17	MX-1: 28, 29

Quality Control Testing

- MEF SQSTM1^{G427R} cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

MEF SQSTM1^{G427R} cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

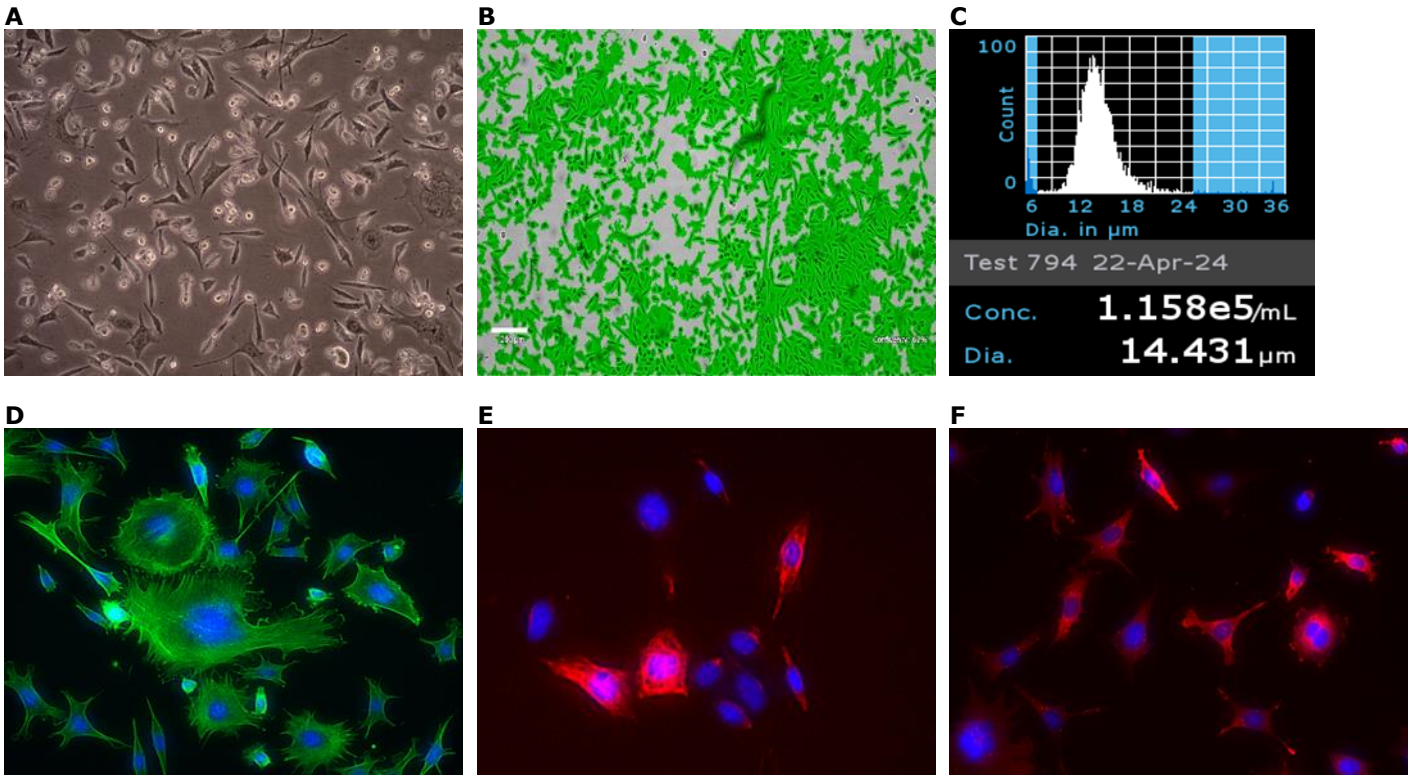

Figure 1. (**A**) Bright-field images of MEF SQSTM1^{G427R} cells two days after thaw in a T25 flask (10X magnification). (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D**) MEF SQSTM1^{G427R} cells stained with Phalloidin-Atto-488 (49409). (**E**) MEF SQSTM1^{G427R} cells express vimentin (SAB4200761). (**F**) MEF SQSTM1^{G427R} cells express SQSTM1 protein (HPA064165). The G427R mutation does not affect the ability to tag SQSTM1 protein with antibody.

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   MEF SQSTM1^{G427R} cells are thawed and expanded in MEF SQSTM1^{G427R} Expansion Medium comprising of DMEM (D5796) containing 10% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Penicillin/Streptomycin (P4333) (optional).
2. Remove the vial of frozen MEF SQSTM1^{G427R} cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   Important: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of MEF SQSTM1^{G427R} Expansion Medium (Step 1 above) to the 15 mL conical tube.

   Important: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   Important: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of MEF SQSTM1^{G427R} Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. MEF SQSTM1^{G427R} cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the tissue culture flask containing the 80-85% confluent layer of MEF SQSTM1^{G427R} cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of MEF SQSTM1^{G427R} Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of MEF SQSTM1^{G427R} cell medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    Important: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

MEF SQSTM1^{G427R} cells may be frozen in MEF SQSTM1^{G427R} Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Deng Z, Lim J, Wang Q, Purtell K, Wu S, Palomo GM, Tan H, Manfredi G, Zhao Y, Peng J, et al. 2019 Jul 30. ALS-FTLD-linked mutations of SQSTM1/p62 disrupt selective autophagy and NFE2L2/NRF2 anti-oxidative stress pathway. Autophagy.:1–15. doi:https://doi.org/10.1080/15548627.2019.1644076.

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Document Template 20306518 Ver 6.0

23118759 Ver 1.0, Rev 03AUG2024, DP