Sigma-Aldrich®

Data Sheet

HEK293 FUS Knockout Human Cell Line

Engineered Cell Line

Cat. # SCC292

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, also known as Charcot or Lou Gehrig's disease) is a progressive neurodegenerative disease resulting in loss of motor control. ALS usually leads to mortality within 5 years of symptom onset.[1] A subset of ALS cases are familial and linked to mutations in a handful of genes, including *FUS* (*fused in sarcoma*). FUS is a ribonucleotide binding protein that plays a role in RNA metabolism and is implicated in DNA repair pathways.[2] Probing the effects of *FUS* point mutations is contributing to our understanding of the cellular mechanisms of ALS and the connections between genetic repair and neurodegeneration.

The HEK293 *FUS* knockout cell line was derived via CRISPR/Cas9-editing of HEK293 cells. Experimental validation of this line confirmed deletion of *FUS* with no off-target effects on expression of related proteins.[2] This *FUS* knockout cell line permits direct study of the effects of *FUS* mutations via transfection of *FUS* variants. The HEK293 *FUS* knockout cell line represents a valuable system for the investigation of familial ALS in a robust cellular model.

Source

HEK293 FUS KO cell line was derived from CRISPR-edited HEK293 cells.[2] The parental HEK293 cell line was derived from cells from an aborted female fetus transfected with sheared adenovirus DNA.[3]

Short Tandem Repeat (STR Profile)

D3S1358:	15,17	D13S317:	12
D7S820:	11, 12	D16S539:	9, 10, 13
vWA:	16, 19	TH01:	9.3
FGA:	23	TPOX:	11
D8S1179:	12, 14	CSF1PO:	12
D21S11:	28, 30.2	Amelogenin:	X
D18S51:	17, 18	Penta D:	9
D5S818:	8	Penta E:	7, 15

Cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

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Quality Control Testing

- HEK293 FUS knockout human cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

HEK293 FUS knockout human 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

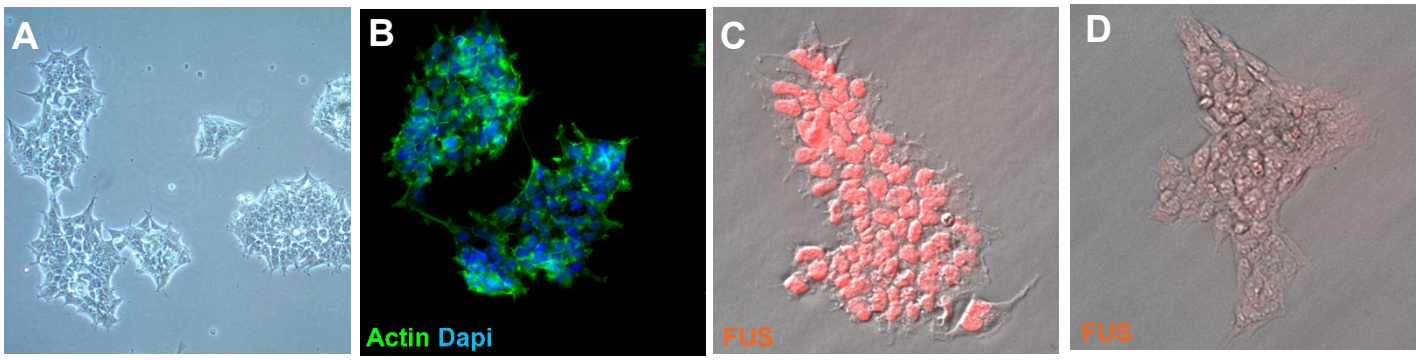

Figure 1. Bright-field image of HEK293 FUS knockout cells two days (**A**) after thaw. Cells express actin (**B**, Sigma P5282). Wild-type HEK293 cells express the RNA-binding protein FUS (**C**, MilliporeSigma MABE1898) while the FUS knock-out cell line does not express FUS (**D**).

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.

   Cells are thawed and expanded in HEK293 FUS KO Expansion Medium comprising DMEM-High Glucose medium (Sigma D6429) containing 10% FBS (e.g. Sigma ES-009-B), 10 mM HEPES (Sigma TMS-003-C) and 2 mM L-Glutamine (Sigma TMS-002-C).
2. Remove the vial of frozen HEK293 FUS KO 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 HEK293 FUS KO 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 HEK293 FUS KO 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. Do not allow the cells to grow to confluency. HEK293 FUS KO cells should be passaged at ~70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of HEK293 FUS KO cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 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 HEK293 FUS KO 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 HEK293 FUS KO Expansion 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.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

HEK293 FUS knockout human cells may be frozen in HEK293 FUS KO Expansion Medium supplemented with 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Zarei S, Carr K, Reiley L, Diaz K, Guerra O et al. *Surg Neurol Int* 2015; 6:171.
2. Wang H, Guo W, Mitra J, Hegde PM, Vandoorne T et al. *Nat Commun* 2018; 9:3683.
3. Graham FL, Smiley J, Russell, WC, Nairn R. *J Gen Virol* 1977; 36(1): 59-74.

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