Data Sheet

S462 Human Peripheral Nerve Sheath Tumor Cell Line

Cancer Cell Line

SCC414

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

Malignant peripheral nerve sheath tumors (MPNSTs) are soft tissue sarcomas that arise from peripheral nerves and result in high rates of local recurrence and hematogenous metastasis. Half of MPNSTs occur in patients with neurofibromatosis type 1 (NF1), a common autosomal dominant multisystem disorder caused by mutations of the NF1 tumor suppressor gene located on chromosome 17q. Loss of function of the intact NF1 allele, due to loss of heterozygosity (LOH) and somatic mutations of the gene, is associated with dermal and plexiform neurofibroma formation. Approximately 10% of NF1 patients develop MPNST, of whom only 21% survive for five years after diagnosis.[1]

Source

S462 is an established human MPNST cell line from a clinically and genetically well-characterized NF1 patient, with a nonsense germline mutation (6792C>A) in exon 37 of the NF1 gene, LOH of the NF1 gene, LOH of the p53 gene, and a homozygous missense mutation (329G>C, Arg>Pro) in exon 4 of the p53 gene.[1]

As a verified MPNST cell line, S462 is a valuable tool for the further investigation of the biology and pathogenesis of this malignancy[2,3] as well as for *in vitro* pharmacologic studies essential for the development of new therapies.[4,5,6,7]

Short Tandem Repeat (STR Profile)

D3S1358:	14, 17	D13S317:	12
D7S820:	8, 10	D16S539:	13
vWA:	19	TH01:	8
FGA:	20	TPOX:	8
D8S1179:	10, 12	CSF1PO:	12, 13
D21S11:	29, 31	Amelogenin:	X
D18S51:	16	Penta D:	9, 11
D5S818:	12, 13	Penta E:	11

Cancer 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.

Quality Control Testing

- S462 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

S462 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

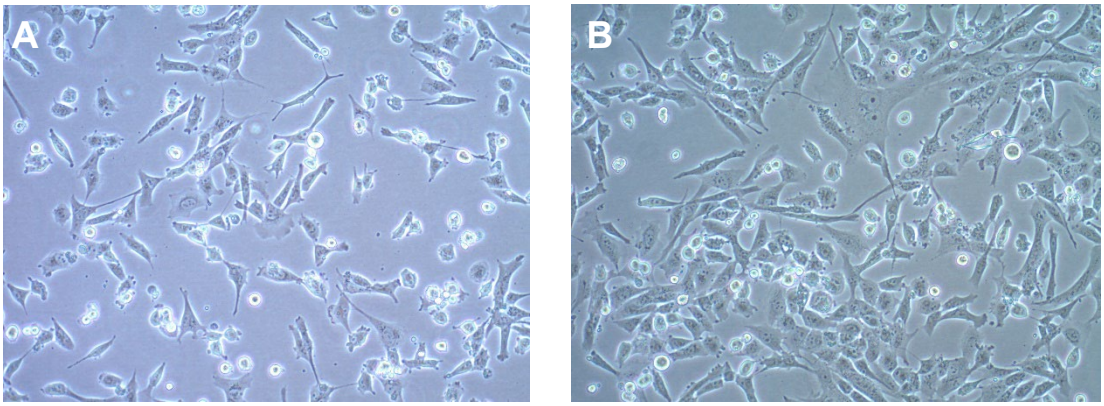

Figure 1. Bright-field images of S462 cells in culture one (**A**) and two days (**B**) after thawing in a T75 flask.

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 S462 Expansion Medium comprising High Glucose DMEM containing L-glutamine and Sodium Pyruvate (Cat. No. D6429), and 10% FBS (Cat. No. ES-00B).

2. Remove the vial of frozen S462 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 S462 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 S462 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. S462 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 S462 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase™ reagent 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 S462 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 S462 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

S462 Human Peripheral Nerve Sheath Tumor Cells may be frozen in S462 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Frahm S, et al. *Neurobiol Dis.* 2004; 16(1): 85-91.
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3. Demestre M, Messerli SM, Celli N, Shahhossini M, Kluwe L, Mautner V, Maruta H. *Phytother. Res.* 2009; 23(2): 226–230.
4. Spyra M, Kluwe L, Hagel C, Nguyen R, Panse J, Kurtz A, Mautner VF, Rabkin SD, Demestre M. *PLoS One* 2011; 6(6): e21099.
5. Reuss DE, Mucha J, Hagenlocher C, Ehemann V, Kluwe L, Mautner V, von Deimling A. *PLoS One* 2013; 8(2): e57152.
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7. Kluwe L, Jiang W, Alster I, Hanken H. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. 2016; 160(1):64-69.

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