

Chordoma Foundation

Cell Line Validation

7316-4062S

Cell morphology, growth and Brachyury
expression analysis

December 20, 2021

Validation Report

Cell line: 7316-4062S

Growth conditions

Media: 4:1 IMDM : RPMI1640 +0% FCS, L-glutamine, Pen/Strep

Or

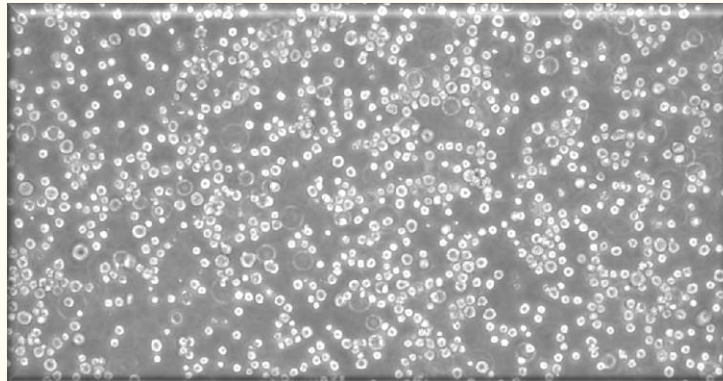
D-MEM/ F12, 0% FCS, Glutamax, Pen/Strep, B27 Supplement Minus Vitamin A (ThermoFisher 12587-010 2%), N2 supplement (ThermoFisher 175020-48 1%), Recombinant Human EGF (Shenandoah 100-26 20 ng/mL), Recombinant Human FGF-basic (Shenandoah 100-146 20ng/mL); Heparin (Stemcell H3149 2ug/ml)

- Change medium up to twice a week

Percentage viable cells after thawing: ~80%

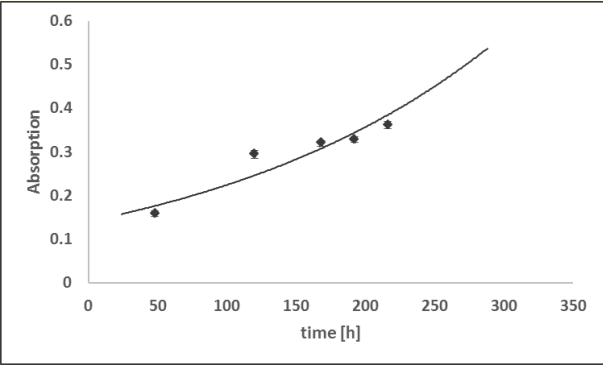
Morphology¹:

rounded cells in suspension



Cells 24 hours post thawing

Population doubling time²:
approx. 6-7 days

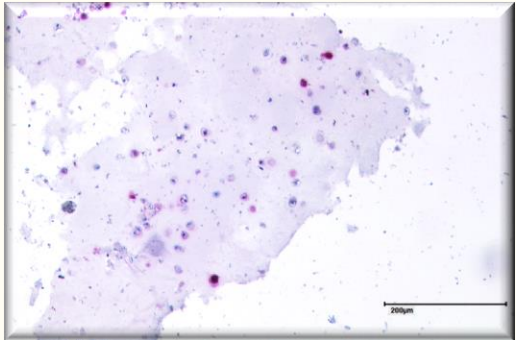


STR profile:

	AMEL	D13S317	D7S820	D16S539	Penta E	TH01	D18S51	D3S1358	D8S1179	TPOX	CSF1PO	Penta D
7316-4062S	X	11 12	8 14	11 13	7 11	6 8	12	14 16	10 13	8	10 11	12 13

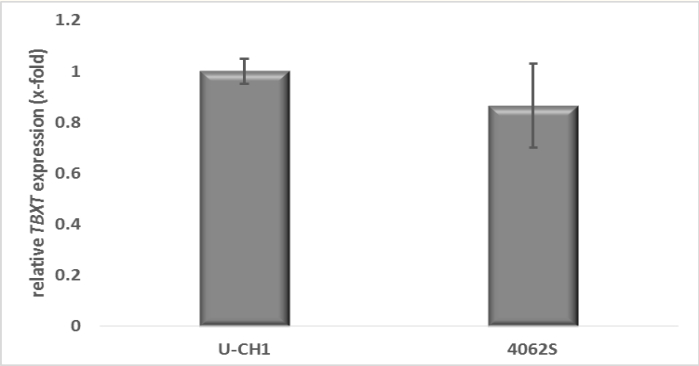
Brachyury expression:

Immunocytology³



~50% of the cells show clear positivity for Brachyury,
~50% show (very) weak positivity

mRNA levels (qPCR detection⁴)



0.9-fold expressed compared to U-CH1

Validation result: The cell line meets the criteria for being a chordoma cell line.

1. Cell morphology was monitored and documented using an invert phase contrast microscope. Typical physaliferous morphology of the cells may vary between different cell lines.
2. Population doubling was estimated by seeding the cells in recommended densities in 96 well plates. Measurement of viable cells was performed using MTS assays at several time points (n=6 per time point).
3. Nuclear positivity for Brachyury was tested using standard immunocytochemistry sections of FFPE cell blocks were used. Stainings were performed using a rabbit monoclonal Brachyury antibody, anti rabbit antibodies and either red or pink or brown dyes.
4. Relative *T* gene expression was calculated using the $\Delta\Delta CT$ method (fold change in relation to *T* expression of U-CH1).