

Cell Line Validation

U-CH22

Cell morphology, growth and Brachyury
expression analysis

02/04/2025

CELL LINE RECEIVING INFORMATION

Format Received	Date Received	Condition	Quantity	Passage	Initial Cell Count	Initial Cell Viability (%)
Frozen Vials	February 24th, 2025	Equivalent to 100% confluent 25cm ² flask	20	p59	n.d. (approx. 2-3 million)	>95%

CELL GROWTH CONDITIONS

GROWTH CONDITIONS

Media: 4:1 IMDM/RPMI 1640 + 10% FBS + Pen/Strep + l-glutamine

Coating: Standard flasks for adherent cells

Passaging: Passage when ~95-100% confluent (1:4 max)

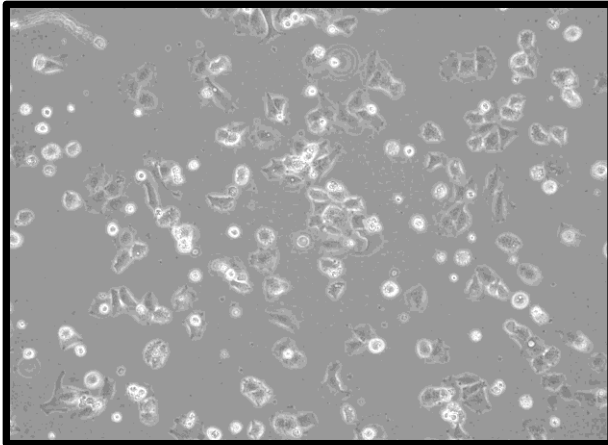
Media Change Frequency: approx. every 3 days (depending on cell density)

Percentage Viable Cells After Thawing: >95%

MORPHOLOGY

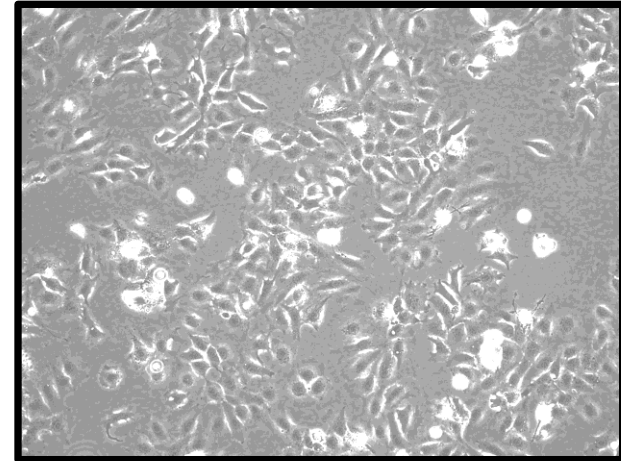
MORPHOLOGY:

“epithelial” shaped, barely physaliferous



CELLS 24 HOUR POST THAWING
(~70% ADHERENT)

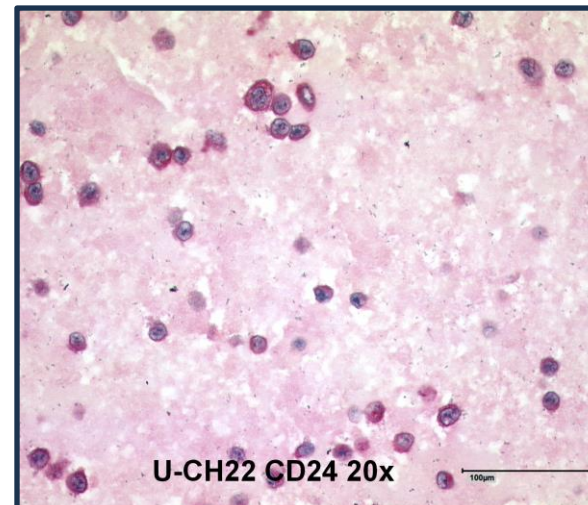
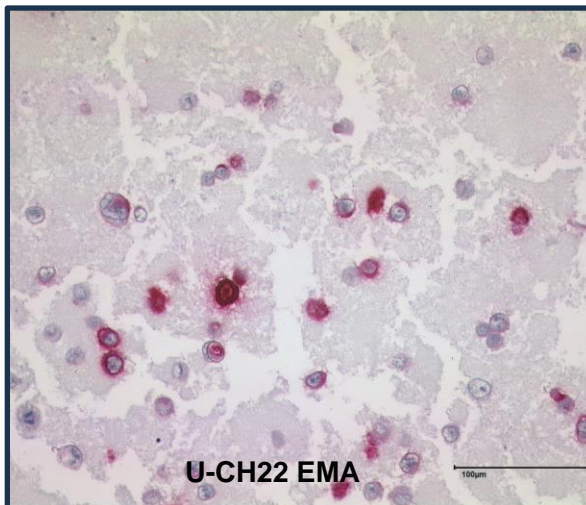
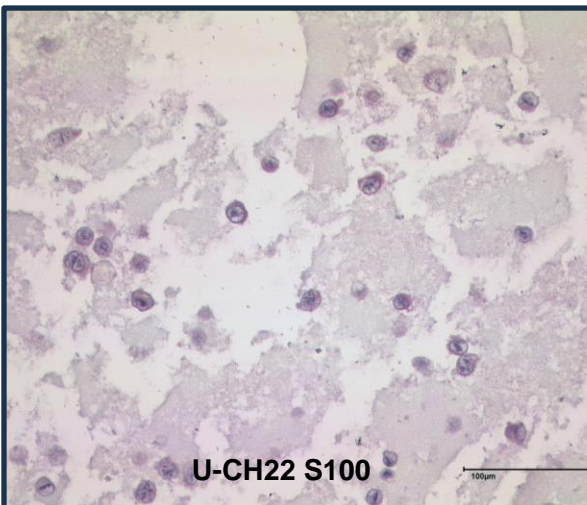
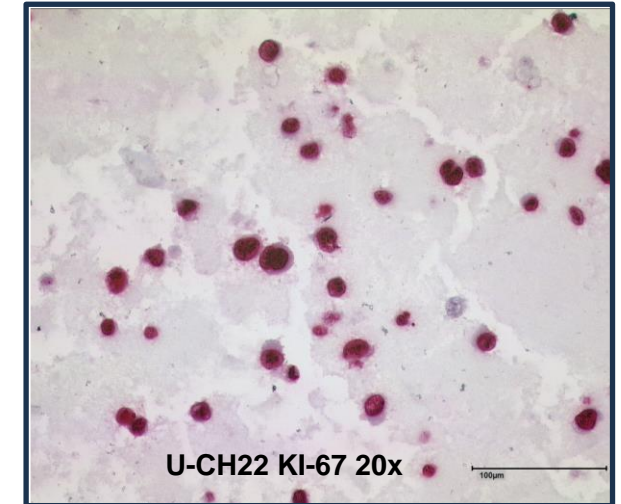
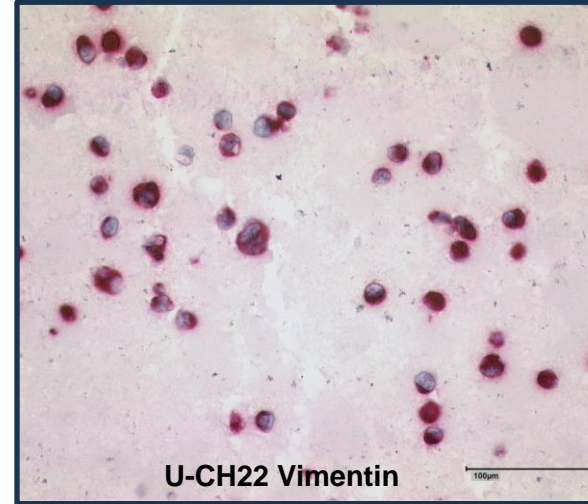
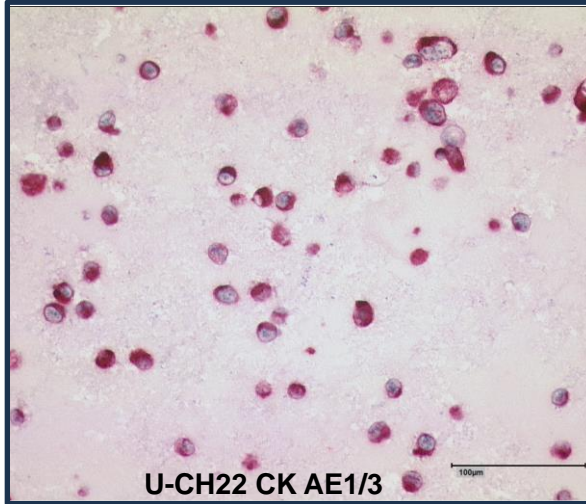
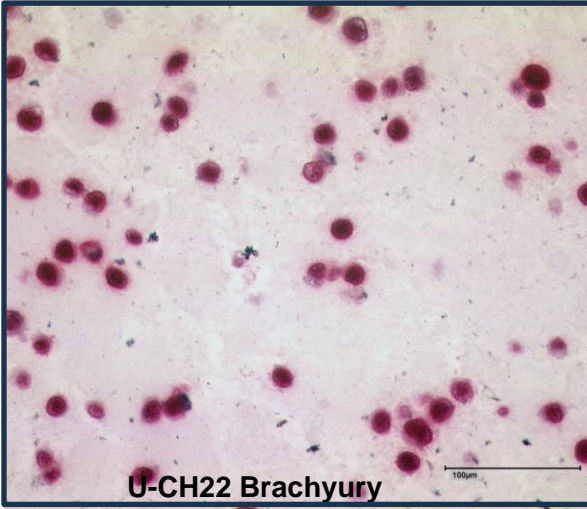
barely physaliferous



CELLS 3 DAYS POST THAWING

CELL LINE ICC VALIDATION

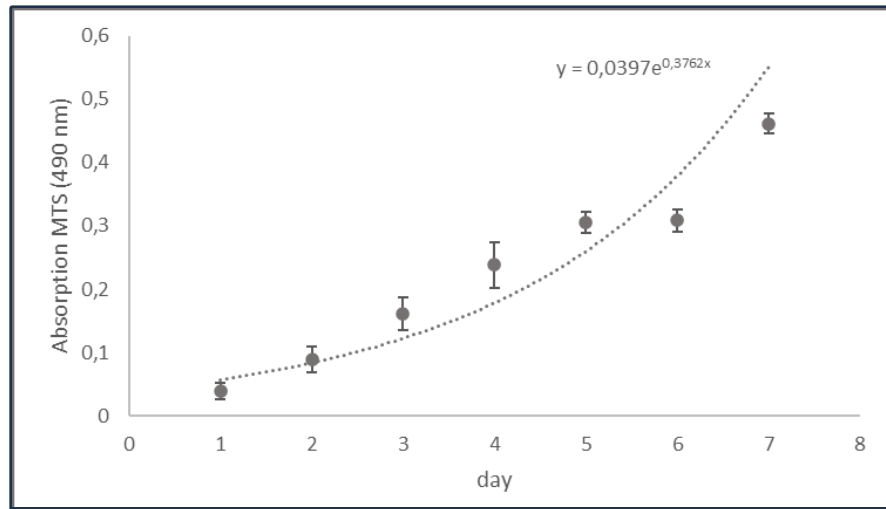
Immunocytochemistry of a chordoma marker panel (Brachyury, pan-cytokeratin, Vimentin, EMA, S100 protein, CD24 and KI-67) in U-CH22



Description: U-CH22 cells show a strong positivity for Brachyury, pan-cytokeratin, vimentin, a questionable / very weak positivity for S100 protein, a partial positivity for EMA, and medium positivity for CD24. KI-67 index is >90%.

STR ANALYSIS

- POPULATION DOUBLING TIME: 2-3 days



Cell growth rates were calculated from an actively growing culture. Growth rates might be slower when calculated from a fresh thaw.

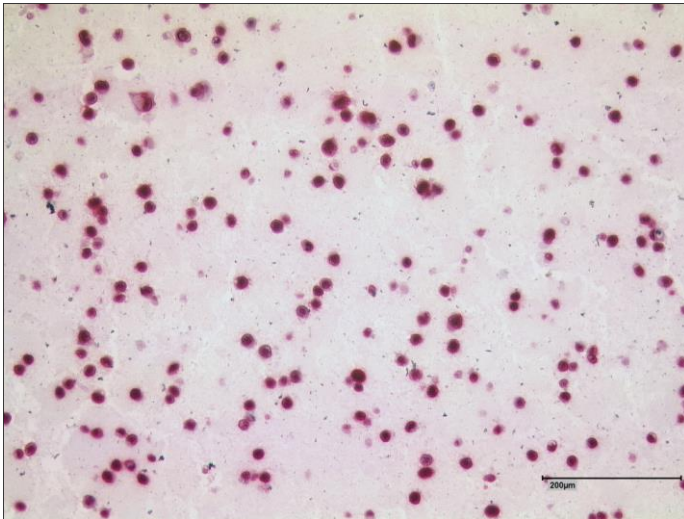
- STR PROFILE:

	Amel	D13S317		D7S820		D16S539		Penta E		TH01		D18S51		D3S1358		D8S1179		TPOX		CSF1PO		Penta D	
U-CH22	X	8	10	8		9	12	10	20	9	9.3	15	16	(15)	16	9	13	9	11	11	12	9	

BRACHYURY EXPRESSION

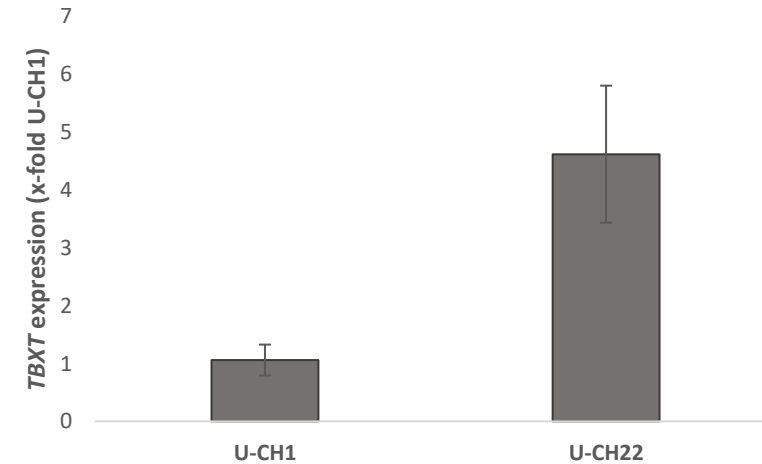
CELL LINE: U-CH22

BRACHYURY EXPRESSION: IMMUNOCYTOLOGY



~100% OF CELLS THAT SHOW POSITIVITY FOR BRACHYURY

mRNA LEVELS (qPCR DETECTION)



CELLS 7 DAYS POST THAWING

CELL LINE VALIDATION RESULTS

TEST	SPECIFICATION	RESULTS
CELL GROWTH	IMMORTILIZED	DOUBLE TIME = 2-3 days
STR ANALYSIS	HUMAN, UNIQUE	PASS
ICC VALIDATION	SIGNAL IN NUCLEUS	PASS
PCR VALIDATION	EXPRESSING BRACHYURY	PASS

VALIDATION RESULT: The cell line meets the criteria for being a chordoma cell line.

CELL LOT GENERATED

STOCK LOT #	
CELLS PER VIAL	n.d. approx. 2-3 million
LOT VIABILITY	>95%
PASSAGES	p. 59