

Conditionally Immortalized Human Umbilical Cord-Derived Mesenchymal Stem Cells

Catalog Number: CIHU001

Cell Quantity: 500,000 cells per vial

Format: Cryopreserved (liquid nitrogen)

Product Description

Conditionally Immortalized Human Umbilical Cord-Derived Mesenchymal Stem Cells (ciUC-MSCs), Catalog #CIHU001, are generated from primary human umbilical cord Wharton's jelly-derived MSCs. The cells are genetically engineered via lentiviral transduction to express Simian Virus 40 Large T antigen (SV40 LT) under the control of a **TetON inducible system**.

In the presence of doxycycline (Dox), SV40 LT expression is activated, enabling reversible cell cycle immortalization. In the absence of Dox, SV40 LT expression is silenced, and cells return to a finite replication capacity. Following transduction, cells were selected with **Puromycin** to ensure stable integration of the transgene.

Morphologically, ciUC-MSCs exhibit characteristic **spindle-shaped, fibroblast-like** appearance and maintain typical mesenchymal stem cell surface marker expression.

Morphology

Parameter	Description
Shape	Spindle-shaped, elongated
Appearance	Fibroblast-like, adherent
Growth pattern	Whorl-like or parallel arrangement at confluence
Adherence	Strongly adherent to tissue culture-treated plastic

Figure note: Cells show typical MSC morphology with uniform spindle shape, and no epithelial-like cobblestone areas.

Immortalization System

Component	Details
Transgene	SV40 Large T antigen (SV40 LT)
Promoter	Inducible TetON (TRE3G promoter)
Inducer	Doxycycline (1–2 µg/mL)
Selection marker	Puromycin resistance (PAC gene)
Selection condition	1–2 µg/mL puromycin for 3–5 days post-transduction
Immortalization	Conditional (reversible upon Dox removal)

Note: Maintain cells in culture medium containing doxycycline to sustain proliferation. Remove doxycycline to induce growth arrest and differentiation potential.

Expression Markers

The following markers have been validated by flow cytometry, immunocytochemistry, and/or qRT-PCR.

Mesenchymal Stem Cell Markers (Positive)

Marker	Expression Level	Validation Method
CD73	High (>95%)	Flow cytometry
CD90	High (>95%)	Flow cytometry
CD105	Moderate-to-High (>90%)	Flow cytometry
CD44	High (>95%)	Flow cytometry
CD29	High (>95%)	Flow cytometry
CD166	Moderate (>85%)	Flow cytometry

Negative Markers (Hematopoietic / Endothelial)

Marker	Expression	Validation Method
CD34	Negative (<2%)	Flow cytometry
CD45	Negative (<2%)	Flow cytometry

Marker	Expression	Validation Method
CD14	Negative (<2%)	Flow cytometry
CD31	Negative (<2%)	Flow cytometry
HLA-DR	Negative (<2%)	Flow cytometry

Immortalization/Inducible Markers

Marker	Condition	Expression
SV40 LT	+ Doxycycline	High (nuclear)
SV40 LT	– Doxycycline	Undetectable (within 5–7 days)
Puromycin resistance (PAC)	Constitutive	Stable (due to IRES or separate promoter)

Pluripotency / Proliferation Markers (in Dox+ condition)

Marker	Status
Ki-67	Positive (>90%)
PCNA	Positive
Telomerase activity	Detectable (hTERT upregulation via SV40 LT)



Differentiation Potential Markers (after Dox withdrawal)

Upon doxycycline removal and culture in lineage-specific media, cells express:

Lineage	Marker	Induction
Osteogenic	Alkaline phosphatase, Osteocalcin, Runx2	Yes
Adipogenic	PPAR γ , FABP4, Oil Red O+ lipid droplets	Yes
Chondrogenic	Aggrecan, Collagen type II, SOX9	Yes

Storage and Handling

Parameter	Instruction
Storage temperature	Liquid nitrogen vapor phase (< -135°C)
Shipping condition	Dry ice
Shelf life	Stable for 2 years from date of manufacture when stored properly
Thawing	Rapid thaw in 37°C water bath (<2 min)
Recommended seeding density	10,000–15,000 cells/cm ² post-thaw



Parameter	Instruction
Medium	MSC growth medium + 1–2 µg/mL doxycycline + 1 µg/mL puromycin (optional for maintenance)

Quality Control

Test	Result
Viability post-thaw	>90% (by Trypan blue exclusion)
Mycoplasma	Negative
Bacterial/fungal sterility	Negative
Human pathogens (HIV, HBV, HCV, HTLV)	Negative
Identity	Confirmed by SV40 LT PCR and CD73/CD90/CD105 flow cytometry
Inducibility	>100-fold increase in population doublings with Dox vs. without Dox over 10 days

Intended Use

For **research use only (RUO)**. Not for diagnostic or therapeutic use in humans or animals. Suitable for:

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- Long-term expansion of UC-MSCs without senescence
- Drug screening and toxicity testing
- MSC differentiation studies (with Dox withdrawal)
- Gene function studies in an MSC background

Ordering and Contact Information

Product	Catalog#	Format
Conditionally Immortalized Human UC-MSCs	CIHU001	500,000 cells/vial
Doxycycline (10 mg/mL sterile)	CIHU-DOX	1 mL
Puromycin (10 mg/mL)	CIHU-PURO	1 mL