



Data Sheet

Conditionally Immortalized Human Adipose Tissue-Derived Mesenchymal Stem Cells

Catalog Number: CIHA001

Cell Quantity: 500,000 cells per vial

Viable Cell Recovery: >90% post-thaw (upon first passage after cryopreservation)

Biosafety Level: BSL-2 (with appropriate lentiviral vector handling precautions)

Product Description

Conditionally Immortalized Human Adipose Tissue-Derived Mesenchymal Stem Cells (CIHA001) are generated from primary human adipose tissue-derived MSCs (hAD-MSCs) via lentiviral transduction of the Simian Virus 40 Large T antigen (SV40 LT) under the control of a Tetracycline-ON (TetON) inducible system.

Key features:

- **Conditional immortalization:** SV40 LT expression is induced only in the presence of doxycycline (Dox). In the absence of Dox, cells exit the cell cycle and re-acquire quiescent MSC characteristics.
- **Selection:** Cells were selected with puromycin to ensure stable integration of the transgene.
- **Morphology:** Spindle-shaped, fibroblast-like cells, forming whorl-like patterns at confluence.

- **Differentiation capacity:** Retain trilineage differentiation potential (adipogenic, osteogenic, chondrogenic) under non-permissive (Dox-free) conditions following expansion in permissive conditions.

Cell Morphology

Parameter	Observation
Shape	Spindle-shaped, elongated, fibroblast-like
Adherence	Strongly adherent to tissue culture-treated plastic
Cytoplasm	Granular, with phase-bright appearance
Nucleus	Single, oval, with 1–2 prominent nucleoli
Growth pattern	Parallel alignment or swirling whorls at high density

Inducible System Overview

- **Promoter:** Inducible TetON (TRE3GS promoter)
- **Transgene:** SV40 Large T antigen (SV40 LT)
- **Inducer:** Doxycycline (Dox) or tetracycline
 - **Permissive conditions (+Dox):** SV40 LT expressed → active proliferation.
 - **Non-permissive conditions (-Dox):** SV40 LT expression silenced → cell cycle arrest, gain of quiescent MSC phenotype.
- **Selection marker:** Puromycin resistance (from bicistronic or co-transduced cassette).

Expression Markers (Characterization Data)

The cells have been validated by flow cytometry, immunocytochemistry, and/or RT-qPCR.

Mesenchymal Stem Cell Markers (Positive in +/- Dox, except where noted)

Marker	Expression	Notes
CD73	Positive (high)	>95%
CD90	Positive (high)	>95%
CD105	Positive (moderate)	>90%
CD44	Positive	>95%
CD29	Positive	>90%
CD166	Positive	>90%

Negative Markers (Hematopoietic/Endothelial)

Marker	Expression	Notes
CD34	Negative	<2%
CD45	Negative	<2%
CD14	Negative	<2%
CD31	Negative	<2%

Marker	Expression	Notes
HLA-DR	Negative	<1%

Inducible Transgene (SV40 LT)

Condition	SV40 LT mRNA	SV40 LT Protein
+ Doxycycline (1–2 µg/mL)	High expression	Nuclear-positive (by ICC)
- Doxycycline (0 µg/mL, 48–72h)	Undetectable	Negative

Proliferation & Immortalization Markers (under +Dox)

Marker	Expression
Ki67	>80% nuclear-positive
PCNA	Upregulated vs primary MSCs
Telomerase activity (hTERT)	Low to absent (endogenous) – SV40 LT extends lifespan but does not reactivate telomerase significantly



Quality Control

Test	Result
Sterility (bacteria/fungi)	Negative
Mycoplasma	Negative (PCR or luminescence)
Viral titer (lentiviral vector)	Undetectable (no replication-competent lentivirus)
Identity (STR profiling)	Matches primary donor source (data available upon request)
Viability post-thaw	$\geq 90\%$
Inducibility (SV40 LT off/on ratio)	>50-fold change by qPCR
Trilineage differentiation (in -Dox)	Positive for Oil Red O, Alizarin Red, Alcian Blue

Storage and Handling

- **Shipping condition:** Dry ice or vapor phase liquid nitrogen.
- **Storage upon receipt:** Immediately transfer to liquid nitrogen vapor phase (-150°C or colder).
- **Do not store at -80°C for extended periods (>2 weeks).**
- **Recommended thawing medium:** Complete growth medium (DMEM low glucose + 15% FBS + L-glutamine + 1% P/S) + 1 µg/mL Doxycycline for first 2 passages.



Recommended Culture Protocol (Brief)

Thawing (Day 0):

1. Thaw vial in 37°C water bath (≤ 2 min).
2. Transfer cells to 10 mL warm **expansion medium**: DMEM (low glucose) + 15% FBS + GlutaMAX + 1 $\mu\text{g}/\text{mL}$ Doxycycline + 0.5 $\mu\text{g}/\text{mL}$ Puromycin (for maintenance of selection).
3. Centrifuge at 300 $\times g$ for 5 min, resuspend, and plate in a T-25 flask.

Expansion (Permissive, +Dox):

- Change medium every 2–3 days.
- Passage at 80–90% confluency (1:3 to 1:4 split).

Differentiation (Non-permissive, -Dox):

- Wash cells 2 \times with PBS, culture in differentiation medium without Dox for 48h prior to induction to silence SV40 LT.

Applications

- Long-term in vitro studies of adipogenesis, osteogenesis, chondrogenesis.
- Drug screening for metabolic or musculoskeletal targets.
- Gene function studies with reversible proliferation control.
- Co-culture systems requiring expandable stromal cells.



Limitations and Warnings

- **Not for human therapeutic use.** For research use only (RUO).
- SV40 LT is a viral oncogene; handle as genetically modified organism (GMO) per institutional guidelines.
- Doxycycline toxicity may occur above 2 µg/mL; optimize for specific assays.
- Loss of differentiation capacity may occur if maintained >20 passages under +Dox.

Ordering Information

Product	Catalog #	Quantity
Conditionally Immortalized Human Adipose MSC	CIHA001	500,000 cells/vial
Expansion Medium Kit (with Dox + Puro)	CIHA-MED	500 mL
Doxycycline Hydrochloride (1000×)	DOX-001	1 mg/mL, 1 mL

IMPORTANT: This warranty is valid for one month from the original purchase date.