Cell name: iAdMSC - Bmi-1/hTERT

Immortalized human adipose tissue-derived mesenchymal stem cells

Cat. No. MSC-001

Product description

iAdMSC-Bmi-1/hTERT cells were established by lentiviral transduction of murine Bmi-1 and human telomerase reverse transcriptase (hTERT) into human primary adipose tissue-derived mesenchymal stem cells (AdMSCs). iAdMSC-Bmi-1/hTERT was maintained for over 140 population doublings (PDs) without major changes and may thus be regarded as immortal [1].

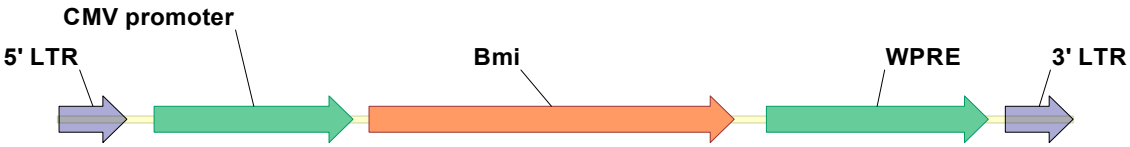
Transgene construct information

Expression cassette type

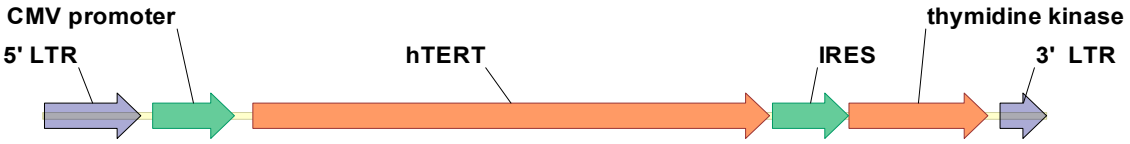
Single-promoter, monocistronic (Bmi-1); single-promoter, bicistronic (hTERT)

Expression cassette map

Bmi-1



hTERT



Expression cassette features

Element	Type	Species	RefSeq	Mutation / Discrepancy
Bmi-1				
CMV	promoter, viral, constitutive	-	-	-
Bmi-1	CDS	Mus musculus	NM_007552.4	-
hTERT				
CMV	promoter, viral, constitutive	-	-	-
hTERT	CDS	Homo sapiens	NM_198253.2	-
IRES	internal ribosomal entry site	Encephalomyocarditis virus	-	-
Thymidine kinase	CDS	Human herpesvirus	AF057310.1	-

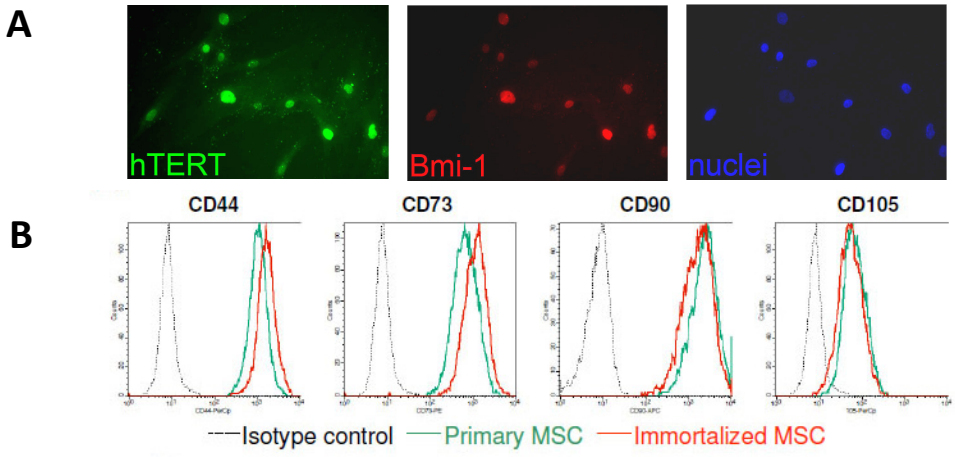
hTERT is the catalytic subunit of the human telomerase complex. In the absence of hTERT, telomeres shorten during cell division because the DNA replication complex cannot completely copy telomeric DNA. Cellular senescence and growth arrest are proposed to occur when telomeres in one or more chromosomes reach a critical length. Supporting this hypothesis is the presence of hTERT and stable telomere lengths in germ cells and most cancer cells. Furthermore, ectopic expression of hTERT leads to telomere elongation and extended life-span in a number of cell types, including fibroblasts, retinal pigment cells, and endothelial cells. Human mesenchymal stem cells have been successfully immortalized by retroviral transduction of hTERT [2]. However, several reports suggest that hTERT alone may be insufficient for the immortalization of human primary cells [3].

The polycomb group protein **Bmi-1** plays a critical role in the self-renewal of adult stem cells, and Bmi-1 is elevated in many types of cancer. Bmi-1 has no known enzymatic activity, but serves as the key regulatory component of the PRC1 complex (polycomb repressive complex-1). This complex influences chromatin structure and regulates transcriptional activity of a number of important loci including the INK4a locus which encodes the tumor suppressor proteins $p16^{INK4a}$ and $p14^{ARF}$ [4]. Overexpressed Bmi-1 may reactivate endogenous telomerase, and it has been shown to immortalize human cells in combination with hTERT [3].

Cell culture characteristics

Source	Lipoaspirate from a 30-year-old healthy female donor
Morphology	Fibroblastic, mesenchymal stem cell-like
Growth properties	Adherent
Culture conditions	Culture cells at 37°C in humidified atmosphere with 5% CO_2. The base medium for this cell line is D-MEM : F-12 1:1 mixture. To make the complete growth medium, add 10% v/v fetal bovine serum, 2 mM L-glutamine, and 50 µg/mL gentamicin. For more rapid proliferation, the growth medium may optionally be supplemented with 1-5 ng/mL FGF-2.
Subculturing	Remove culture medium and detach cells by treating with 0.04 mL/cm^2 of 0.25% trypsin / 1 mM EDTA solution for 10 minutes at 37°C. Add 5x volume of complete medium to neutralize trypsin-EDTA, pellet cells at 250 x *g*, discard supernatant, and resuspend in culture medium. Subcultivate in a ratio of 1:2-1:3 once a week. Recommended plating density: $4x10^3$ cells/cm^2.
Preservation	Freeze Medium: Complete growth medium with 10% DMSO. Storage Temperature: Liquid nitrogen vapour phase.
Population doubling time	5-7 days
Sterility testing	Mycoplasma: negative

Validation results



A Fluorescence immunolabeling of hTERT (Epitomics) and Bmi-1 (Merck / Millipore). Nuclei are stained with DAPI.

B iAdMSC-Bmi-1/hTERT was positive for the mesenchymal stem cell markers CD73, CD44, CD90, and CD105 at PD109, and negative for hematopoietic markers at PD50 (not shown).

References

[1] Tátrai et al. Combined introduction of Bmi-1 and hTERT immortalizes human adipose tissue-derived stromal cells with low risk of transformation. Biochem Biophys Res Commun. 2012 May 25;422(1):28-35.

[2] Simonsen et al. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol. 2002 Jun;20(6):592-6.

[3] Haga et al. Efficient immortalization of primary human cells by p16INK4a-specific short hairpin RNA or Bmi-1, combined with introduction of hTERT. Cancer Sci. 2007 Feb;98(2):147-54.

[4] Cao et al. BMI1 as a novel target for drug discovery in cancer. J Cell Biochem. 2011 Oct;112(10):2729-41.

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