



Applied Biological Materials Inc.

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Immortalized Human Bone Marrow Mesenchymal Cells – hTERT

Cat.No.

T0523

Unit

1x10⁶ cells / 1.0 ml

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Name	Immortalized Human Bone Marrow Mesenchymal Cells – hTERT
Description	<p>The Bone Marrow Mesenchymal Cells are important for the growth and differentiation of primitive haematopoietic cells, as well as for maintenance of leukaemic cells. The immortalized cell was created by infecting primary mesenchymal cells with retrovirus containing hTERT and GFP genes separated by IRES under the control of MSCV LTR (pCIneo-hEST2-HA).</p> <p>The Immortalized Bone Marrow Mesenchymal cells can support growth of leukaemic cell lines 380, REH, OP-1 and 697, in addition to support the expansion of CD34+ cells. This cell line is useful not only in supporting the growth of haematopoietic cells, but can also be used in studies concerning bone marrow microenvironment and environmental cues that regulate haematopoiesis.</p>
Organism	Human (H. sapiens)
Tissue	Bone Marrow
Donor History	Not disclosed
Growth Properties	Adherent, spindle-shaped
Cell Type	Immortalized Cells
Unit	1x10 ⁶ cells / 1.0 ml
Storage Condition	Vapor phase of liquid nitrogen, or below -130°C.
Shipping Conditions	Ship with dry ice.
Product Format	Frozen
Intended Use	This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.
BioSafety	II
Certificate of Analysis	For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.abmgood.com .
Growth Conditions	<p>Use of PriCoat™ T25 Flasks (G299) or Applied Cell Extracellular Matrix (G422) is required for cell adhesion to the culture vessels. PriGrow II (TM002) + 10% FBS(Regular*) + 10⁻⁶ mol/L hydrocortisone + 1% Penicillin/Streptomycin Solution (G255), 37.0°C, 5% CO₂</p> <p>*Do not heat-inactivate</p>

Unpacking and Storage Instructions	<ol style="list-style-type: none"> 1. Visually examine the packaging containers for signs of leakage or breakage. 2. Immediately transfer frozen cells from dry ice packaging to a temperature below -130°C, preferably in liquid nitrogen vapor phase storage, until ready for use. <p>To ensure the highest level of viability, thaw the vial and initiate culture as soon as possible upon receipt. If continued storage is desired, the vial should only be stored below -130°C or in liquid nitrogen vapor phase. Do not store at -70°C, as it will result in loss of viability.</p>
Thawing Protocol	<ol style="list-style-type: none"> 1. Thaw cells quickly in a 37°C water bath while agitating gently (maximum 2 minutes). The vial cap should be kept above the water level to minimize the risk of contamination. 2. Decontaminate the vial by spraying and wiping the exterior of the vial with 70% ethanol. From this point onwards, all operations should be strictly carried out inside a biological safety cabinet using aseptic conditions. 3. Transfer the cell suspension into a 15ml sterile conical tube containing 5ml of pre-warmed, complete growth media. Centrifuge cells at 125xg for 5–7 minutes. 4. Aspirate the supernatant without disturbing the cell pellet. Re-suspend the cell pellet in the recommended pre-warmed, complete growth media and dispense into a T25 culture flask. 5. Incubate the cells at the recommended conditions.
Subculture Protocol	<p>Volumes given below are for a T75 flask; proportionally increase or decrease the volume as required per culture vessel size. Subculture cells once the culture vessel is 80% confluent.</p> <ol style="list-style-type: none"> 1. Aspirate the culture media, and add 2–3ml of pre-warmed 0.25% Trypsin-EDTA to the culture vessel. 2. Observe the cells under a microscope to confirm detachment (typically within 2–10 minutes). Cells that are difficult to detach can be put in 37°C, for several minutes to facilitate detachment. 3. Neutralize Trypsin-EDTA by adding an equal volume of the complete growth media into the culture vessel. 4. Transfer the culture suspension into a sterile centrifuge tube, and centrifuge at 125xg for 5 minutes. The actual centrifuge duration and speed may vary depending on the cell type. 5. Aspirate the supernatant, and re-suspend the pellet with pre-warmed fresh complete growth media. Add appropriate aliquots of the cell suspension to new culture vessels, as desired. 6. Incubate the cells at the recommended conditions.
Cryopreservation	Cryopreservation Medium (TM024), or complete growth media with 10% DMSO.
Seeding Density (cells/cm²)	10,000 – 20,000
Split Ratio	1:2 to 1:3
Population Doubling Time (h)	35 – 45
Immortalization Method	Serial passaging and transduction with recombinant VSV-G retrovirus carrying hTERT and GFP genes
Expression	CD44, CD73, Osteoblasts markers (alkaline phosphatase), Chondrocytes markers (incorporation of toluidine blue dye)

Material Citation	If use of this material results in a scientific publication, please cite the material in the following manner: Applied Biological Materials Inc, Cat. No. T0523.
Warranty	abm warrants that cell lines shall be viable upon initiation of culture for a period of thirty (30) days after shipment and that they shall meet the specifications on the applicable abm Material Product Information sheet, certificate of analysis, and/or catalog description. Such thirty (30) day period is referred to herein as the "Warranty Period".
Disclaimer	<p>1. Sale of this item is subjected to the completion of a Material Transfer Agreement (MTA) by the purchasing individual/institution for each order. If you have any questions regarding this, please contact us at licensing@abmgood.com.</p> <p>2. All test parameters provided in the CoA are conducted using abm's standardized culture system and procedures. The stated values may vary under the end-user's culture conditions. Please verify that the product is suitable for your studies by referencing published papers or ordering RNA (0.5 µg, Cat.# C207, \$450.00) or cell lysate (100 µg, Cat.# C206, \$600.00) to perform preliminary experiments, or alternatively use our Gene Expression Assay Service (Cat# C138). All sales are final.</p> <p>3. We recommend live cell shipments for ease of cell transfer and this option can be requested at the time of ordering. Please note that the end-user will need to evaluate the feasibility of live cell shipment by taking into account the final destination's temperature variation and its geographical location. In addition, we thoroughly test our cell lines for freeze-thaw recovery. If frozen cells were received and not recovered in your lab under the exact, specified conditions (using recommended culture vessel, media, additional supplements, and atmospheric conditions), a live cell replacement is possible at a cost (plus shipping).</p> <p>4. All of abm's cell biology products are for research use ONLY and NOT for therapeutic/diagnostic applications. abm is not liable for any repercussions arising from the use of its cell biology product(s) in therapeutic/diagnostic application(s). Please contact a technical service representative for more information.</p> <p>5. abm makes no warranties or representations as to the accuracy of the information on this site. Citations from literature and provided for informational purposes only. abm does not warrant that such information has been shown to be accurate.</p> <p>6. abm warrants that cell lines shall be viable upon initiation of culture for a period of thirty (30) days after shipment and that they shall meet the specifications on the applicable abm Material Product Information sheet, certificate of analysis, and/or catalog description. Such thirty (30) day period is referred to herein as the "Warranty Period."</p>
Depositor	St. Jude Children's Research Hospital
QC	1) Immunofluorescence and RT-PCR were used to confirm the presence hTERT expression; 2) PCR based TRAP was used to assay telomerase activity; 3) Neo concentration not determined for selection use but cells were sorted for GFP+ populations
Application	Research Use Only.

Caution: This product is for research use only and is not intended for therapeutic or diagnostic applications.

Please contact a technical service representative for more information (1-866-757-2414).