



Applied Biological Materials Inc.

Telephone:1-866-757-2414

Email:info@abmgood.com

Website:www.abmGood.com

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## PARP-1 Knockout Immortalized Mouse Heart Endothelial (HYKO6) Cell Line

<b>Cat.No.</b>	<b>Unit</b>
T3031	1x10 <sup>6</sup> cells / 1.0 ml

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<b>Cat. No.</b>	T3031
<b>Name</b>	PARP-1 Knockout Immortalized Mouse Heart Endothelial (HYKO6) Cell Line
<b>Description</b>	<p>Poly(ADP-ribose) polymerase 1 (PARP-1) is an ADP-ribosylating enzyme that plays a critical role in DNA damage repair, in addition to functioning as a context-specific regulator of transcription factors. Various pathophysiological conditions, such as reperfusion injury, endotoxic shock, diabetes and aging are associated with endothelial cell dysfunction mediated by PARP-1.</p> <p>The PARP-1<sup>-/-</sup> Knockout Immortalized Mouse Heart Endothelial (HYKO6) Cell Line is derived from stable transformation of SV40 into primary 9-10 week old 129/Sv x C57BL/6 mouse heart endothelial cells deficient for PARP-1. This cell line is capable of morphologic differentiation in capillary-like structures (CLS), similar to its primary counterparts, as shown by branching and anastomosing cords of cell networks and tube formation after 12 hours culturing on Matrigel. Moreover, HYKO6 responds to cytokine stimulation and has been shown to exhibit increased VCAM-1 and E-selectin expression after TNF<math>\alpha</math> stimulation, making this cell line an important tool in the elucidation of PARP-1 signaling pathway involved in endothelial cell dysfunction and molecular mechanisms controlling cell gene expression in response to inflammatory stimuli.</p>
<b>Organism</b>	Mouse (M. musculus)
<b>Tissue</b>	Heart
<b>Growth Properties</b>	Adherent, cobblestone
<b>Cell Type</b>	Stable Cell Lines
<b>Unit</b>	1x10 <sup>6</sup> cells / 1.0 ml
<b>Storage Condition</b>	Vapor phase of liquid nitrogen, or below -130°C.
<b>Shipping Conditions</b>	Ship with dry ice.
<b>Product Format</b>	Frozen
<b>Intended Use</b>	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.
<b>BioSafety</b>	II

<b>Certificate of Analysis</b>	For batch-specific test results, refer to the applicable certificate of analysis that can be found at <a href="http://www.abmgood.com">www.abmgood.com</a> .
<b>Growth Conditions</b>	<p><b>For optimal cell culture, we recommend using PriCoat™ T25 Flasks (<a href="#">G299</a>) or coating your preferred vessels with Applied Cell Extracellular Matrix (<a href="#">G422</a>).</b></p> <p>RPMI 1640 Medium (<a href="#">TM503</a>) + 10% FBS(Regular*) + 1% Penicillin/Streptomycin Solution (<a href="#">G255</a>), 37.0°C, 5% CO<sub>2</sub>.</p> <p>*Do not heat-inactivate</p>
<b>Unpacking and Storage Instructions</b>	<ol style="list-style-type: none"> <li>1. Visually examine the packaging containers for signs of leakage or breakage.</li> <li>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.</li> </ol> <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>
<b>Thawing Protocol</b>	<ol style="list-style-type: none"> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>5. Incubate the cells at the recommended conditions.</li> </ol>
<b>Subculture Protocol</b>	<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"> <li>1. Aspirate the culture media, and add 2–3ml of pre-warmed 0.25% Trypsin-EDTA to the culture vessel.</li> <li>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.</li> <li>3. Neutralize Trypsin-EDTA by adding an equal volume of the complete growth media into the culture vessel.</li> <li>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.</li> <li>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.</li> <li>6. Incubate the cells at the recommended conditions.</li> </ol>
<b>Cryopreservation</b>	We recommend using serum-free CryoGuard™ Freezing Media ( <a href="#">TM078</a> ) or, if serum is preferred, Cryopreservation Medium ( <a href="#">TM024</a> ).
<b>Seeding Density (cells/cm<sup>2</sup>)</b>	20,000 – 30,000

<b>Population</b>	30 - 40
<b>Doubling Time (h)</b>	
<b>Expression</b>	CD105, CD31, ICAM-2, VCAM-1, vWF, MHC Class I antigens
<b>Material Citation</b>	If use of this material results in a scientific publication, please cite the material in the following manner: Applied Biological Materials Inc, Cat. No. T3031.
<b>Warranty</b>	<b>abm</b> 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 <b>abm</b> Material Product Information sheet, certificate of analysis, and/or catalog description. Such thirty (30) day period is referred to herein as the "Warranty Period".
<b>Disclaimer</b>	<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 <a href="mailto:licensing@abmgood.com">licensing@abmgood.com</a>.</p> <p>2. All test parameters provided in the CoA are conducted using <b>abm</b>'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 <b>abm</b>'s cell biology products are for research use ONLY and NOT for therapeutic/diagnostic applications. <b>abm</b> 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. <b>abm</b> makes no warranties or representations as to the accuracy of the information on this site. Citations from literature and provided for informational purposes only. <b>abm</b> does not warrant that such information has been shown to be accurate.</p> <p>6. <b>abm</b> 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 <b>abm</b> 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>
<b>Depositor</b>	IMIM
<b>QC</b>	1) Flow cytometry was used to confirm the expression of endothelial cell markers such as CD105, CD31, ICAM-2, VCAM-1 and MHC Class I antigens; 2) Immunofluorescence staining was used to assess the vWF expression; 3) Western blot was used to evaluate the expression of SV40 T-antigen in the immortalized cell line.
<b>Application</b>	Research Use Only.

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**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).*