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## A30P aSYN-IRES-GFP Stable LUHMES Cell Line

<b>Cat.No.</b>	<b>Unit</b>
T6454	1x10 <sup>6</sup> cells / 1.0 ml

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<b>Cat. No.</b>	T6454
<b>Name</b>	A30P aSYN-IRES-GFP Stable LUHMES Cell Line
<b>Description</b>	<p>This cell line, the A30P aSYN-IRES-GFP Stable LUHMES Cell Line, was generated through lentiviral infection. These cells stably express A30P aSyn and are suitable for investigations focusing on neurotoxicity and the identification of targets for the treatment/prevention of neurodegenerative disorders.</p> <p><b>abm</b> also offers: A53T aSYN-IRES-GFP Stable LUHMES Cell Line (<a href="#">T6455</a>), Wild Type aSYN-IRES-GFP Stable LUHMES Cell Line (<a href="#">T6456</a>) IRES-GFP Stable LUHMES Cell Line (<a href="#">T6457</a>)</p>
<b>Organism</b>	Human (H. sapiens)
<b>Tissue</b>	Brain
<b>Donor History</b>	8-week-old fetal human ventral mesencephalon
<b>Growth Properties</b>	Adherent, epithelial
<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>	Grow the cells in culture vessel pre-coated with 50 µg/ml poly-L-ornithine (PLO) (TM062) and 1 µg/ml fibronectin (EMD Millipore; Cat. FC010) in H <sub>2</sub> O for at least 3 hours at 37°C. Do not grow these cells in culture vessels with surface areas equal to or less than 12.5 cm <sup>2</sup> . These cells do not grow in 6-well, 24-well, 48-well, or 96-well plates. The base medium for this cell line is Advanced DMEM/F12 (Gibco;12634010). To make the complete growth medium, add the following

components to the base medium: N2 supplement (ThermoFisher Scientific) to a final concentration of 1X, L-glutamine (G275) to a final concentration of 2 mM, and Recombinant Human FGF2 (Z101455) to a final concentration of 40 ng/ml. Cells may be grown in the presence of 1 µg/ml Tetracycline. Change media every 2-3 days. Do not let media colour change to orange-yellow. The cells will form round clumps instead of a monolayer when stressed. It is recommended to subculture when cells are grown to a cell density of 50-60%. Carbon dioxide (CO<sub>2</sub>): 5%, Temperature: 37.0°C. To subculture the cells, use TrypLE Express. After adding TrypLE Express, incubate the cells at 37.0°C incubator for 2-3 minutes and agitate the culture vessel until 90% of the cells have detached. Immediately neutralize the trypsin using Trypsin Neutralizing Solution (Lonza, Cat. CC-5002). Add Trypsin Neutralizing Solution the same volume of trypsin used. Centrifuge at 200x g for 2-3 minutes. Aspirate supernatant and resuspend cells in complete media. Plate cells into pre-warmed PLO-fibronectin-coated vessels at 37.0°C. Avoid subculturing if the cells appear stressed. Note: If leaving the cells over the weekend (or more than 2 days), make sure to do a high split ratio (1:4 to 1:5). For information regarding cell differentiation, please refer to the Differentiation Protocol PDF under the Documents Tab.

### **Unpacking and Storage Instructions**

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.

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.

### **Thawing Protocol**

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**

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.

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** We recommend using serum-free CryoGuard™ Freezing Media ([TM078](#)).

**Expression** A30P aSyn, GFP, Tetracycline resistance

**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. T6454.

**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**

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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.
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).
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**Depositor** Georg-August-Universität Göttingen

**QC** 1) Western blot; 2) Immunocytochemistry

**Application**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).*