Panomics

Stable Cell Line: 293/AP1-luc

Product Insert

Catalog No. RC0006

Product Description

The 293/AP1-luc cell line is designed for monitoring the activity of AP-1 transcription factor in cell-based assays. The 293/AP-1-luc cell line was obtained by co-transfection of pAP1-luc (Panomics P/N LR0003) and pHyg into human embryonic kidney 293 cells, followed by hygromycin selection. PMA-induced luciferase activity was used to select clones from the hygromycin-resistant cells. These cells maintain a chromosomal integration of a luciferase reporter construct regulated by multiple copies of the AP-1 response element.

Contents and Storage

One vial of 1 x 10^6 cells, at passage 5, in Freezing Media.

IMPORTANT Cells are shipped frozen. If cells are not frozen upon arrival, contact Panomics immediately.

IMPORTANT Place frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Product Warranty

Panomics warrants that cells shall be viable upon shipment from Panomics for a period of thirty days, provided they have been properly stored and handled during this period.

Handling Cells Upon Arrival

We strongly recommend that you propagate the cells, using the provided procedure, as soon as possible. This will ensure the best cell viability and assay performance.

Cell Line Stability

Cells will undergo genotypic changes resulting in reduced responsiveness over time in normal cell culture conditions. Genetic instability is a biological phenomenon that occurs in all stably transfected cells. Therefore, it is critical to prepare an adequate number of frozen stocks at early passages.

Required Cell Culture Media

Component	Supplier	Final Concentration	Initial Growth Media	Complete Growth Media	Serum Free Media	Freezing Media
Dulbecco's Modified Eagle's Medium, 500 mL	ATCC P/N 30-2002	—	✓	✓	✓	✓
Fetal Bovine Serum	ATCC P/N 30-2020	10%	✓	✓	—	✓
Penicillin, 10,000 units/mL; Streptomycin, 10,000 µg/mL	ATCC P/N 30-2300	100 units/mL 100 µg/mL	✓	✓	✓	✓
Hygromycin B[a]	Roche P/N 10843555001	100 µg/mL	—	✓	—	—
DMSO	ATCC P/N 4-X	10%	—	—	—	✓

a. We recommend you do not substitute the vendor because the quality of hygromycin B can vary substantially among vendors.

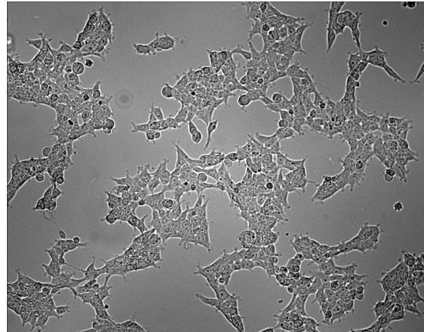
Initial Culture Procedure

The first propagation of cells should be for generating stocks for future use. This ensures the stability and performance of the cells for subsequent experiments.

To thaw cells initially:

Step	Action
1	Prepare culture dish by adding 15 mL of pre-warmed Initial Growth Media to a 100-mm culture dish or T-75 culture flask.
2	Quickly thaw cells in a 37 °C water bath with constant agitation.
3	Clean the outside of the vial with 70% ethanol before opening.
4	Immediately transfer entire contents of the vial to the prepared culture dish. DO NOT pipet cells up and down as this may damage them.
5	Rock the culture dish gently to distribute the cells.
6	Place the culture dish with cells in a humidified incubator at 37 °C and 5% CO_2.
7	After 48 hours, change to Complete Growth Media.
8	Change media every 2–3 days using Complete Growth Media.
9	When cells reach 90% confluency (usually within 1 week), prepare frozen stocks and continue to propagate the rest of the culture for functional assays.

Cell Morphology



293/AP1-Luc cells in culture, passaged once after thaw. These cells display a typical epithelial cell morphology.

Preparing Frozen Stocks

To freeze cells:

Step	Action
1	Detach cells from culture dish according to the “Sub-Culture Procedure.”
2	Resuspend cells at a density of 5 x 10^6 cells/mL in Freezing Media. **Note** A T-75 culture flask typically yields enough cells for preparing two frozen vials.
3	Aliquot 1 mL cells into cryogenic vials (Fisher Scientific P/N 03-374-21).
4	Place vials in a freezing container (VWR P/N 55710-200) and store at –80 °C overnight.
5	Transfer vials to liquid nitrogen for long term storage. If properly stored, cells should remain stable for years.

Sub-Culture Procedure

These cells attach loosely to the culture dish. Pipet gently when changing the media. Sub-culture cells when they have reached about 90% confluency or about every 2–3 days.

This procedure is specific for 100-mm culture dish or T-75 culture flasks. Scale volumes appropriately for other culture vessels.

To sub-culture the cells:

Step	Action
1	Remove culture media from cells by aspiration.
2	Briefly rinse the cells with 5 mL of phosphate-buffered saline (PBS) (ATCC P/N 20-2101), then remove PBS by aspiration.
3	Add 2 mL of 0.25% trypsin/0.53 mM Tris-EDTA solution (ATCC P/N 30-2101) to the culture dish.
4	Let the culture dish stand at room temperature for 2–3 minutes. Confirm detachment by observation with a microscope. Add 10 mL of pre-warmed Complete Growth Media and gently pipet up and down to break up any clumps. **IMPORTANT** To avoid clumping, do not agitate the cells by tapping or shaking the culture dish while waiting for the cells to detach. If cells are difficult to detach, place them at 37 °C for about 5 minutes to facilitate cell detachment.
5	Transfer cells to a 15 mL conical centrifuge tube and centrifuge at 125 x g for 5 minutes to collect the cells.
6	Aspirate the culture media and resuspend cells in 10 mL of pre-warmed Complete Growth Media.
7	Dispense 2 mL of the cell suspension into each new 100-mm culture dish or T-75 flask.
8	Add 13 mL of pre-warmed Complete Growth Media to each culture vessel containing the 2 mL cell suspension.
9	Place in a humidified incubator at 37 °C with 5% CO_2. Change media every 2–3 days and split cells when they reach about 90% confluency.

Quality Assurance

When treated according to the following procedure, these cells displayed a 30-fold induction of luciferase activity by treatment with an AP-1 activator.

Functional assay procedure:

Step	Action
1	Seed cells at 5 x 10^5/well in 1 mL of Initial Growth Media in a 12-well plate.
2	Incubate the culture dish in a humidified incubator at 37 °C and 5% CO_2 for 24 hours to allow cells to recover and attach.
3	Replace media with 1 mL of Serum Free Media. Add PMA to achieve a final concentration of 10 ng/mL to non-control cells.
4	Incubate the culture dish in a humidified incubator at 37 °C and 5% CO_2 for 16 hours.
5	Remove media, add 100 µL of lysis buffer to each well, and assay for luciferase activity according to assay manufacturer's (Promega P/N E1500) recommendations. For more information about this cell line, go to www.panomics.com.

96-Well Assay Procedure

The following procedure should be used as a guideline. You will need to optimize the assay conditions based upon your experimental setup.

To prepare cells for a luciferase assay:

Step	Action
1	Start with cells in log-growth phase. The day before the assay, trypsinize the cells and seed each well of a 96-well plate with 5 x 10^4 cells in 100 µL.
2	Incubate the plate in a humidified incubator at 37 °C with 5% CO_2 overnight.
3	Prepare inducing reagent at the optimal concentration in a 10 µL volume.
4	Add inducing reagent directly to each well and incubate for an appropriate time to produce maximal induction.
5	Remove the media by aspiration and add 50 µL lysis buffer (Promega P/N E1500) to each well.
6	Incubate cells in lysis buffer for 2 minutes at room temperature.
7	Make sure cells are lysed by pipetting up and down (avoid foaming by pipetting gently).
8	Perform one freeze-thaw cycle at –80 °C and room temperature.
9	Gently pipet up and down 2–3 times to mix.
10	Transfer 20 µL of each lysate to a new 96-well plate for the luciferase assay.
11	Add 20 µL of luciferase substrate to each well and gently pipet up and down 2–3 times to mix.
12	Immediately read the plate in a luminometer.

Contacting Panomics

For technical questions, contact our support group by telephone at (877) 726-6642 or by email at techsupport@panomics.com, or visit our website at www.panomics.com.

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