NCI-H1299-EGFP, with G418 resistance & silenced reporter (DKFZ # P-1045)

300272

GENERAL INFORMATION

Description

The NCI-H1299 RFP cells, modified to include a reporter in the DAPK1 gene, are not only useful for studying specific gene activation but also provide a broader understanding of how cells react to epigenetic drugs globally. By using a technique called Cap Analysis of Gene Expression (CAGE), researchers have been able to detail changes in where transcription starts across the genome in response to treatments with DNMTi (DAC), HDACi (SAHA or SB939), or their combinations. This method reveals not just the expected reactivation of the DAPK1 gene but also the emergence of new transcription start sites, called treatment-induced non-annotated TSSs (TINATs), especially under drug treatment. These new start sites are typically located in regions of the genome that do not usually produce proteins and lead to the creation of new RNA molecules that could potentially code for proteins.

Further analysis shows that these new RNA molecules can sometimes merge with existing ones to form what are known as TINAT-exon fusion transcripts. Depending on how these transcripts are spliced, they can translate into new, atypical proteins. This process has been confirmed through laboratory techniques that demonstrate these transcripts can indeed lead to the production of new protein forms. These proteins might interact abnormally within the cell or be recognized as foreign by the immune system, potentially offering new targets for cancer therapy.

The activation of these TINATs involves intricate changes in both DNA methylation and histone modifications, illustrating a complex interaction between these epigenetic factors under drug treatment. Particularly, the combined use of DAC and SB939 shows a greater effect, boosting the expression of these novel transcripts more than when either drug is used alone. Understanding these interactions and their outcomes helps clarify how epigenetic therapies alter cell behavior and opens up possibilities for new cancer treatments that leverage these complex molecular changes.

Tissue	Lung
Disease	Large cell carcinoma
Metastatic site	Primary tumor site (lung)

Applications

Epigenetic therapy research; DAPK1 gene reactivation; DNMTi and HDACi drug response studies; treatment-induced non-annotated TSSs (TINATs); CAGE transcriptomics; fusion transcript analysis; atypical protein production from cryptic promoters; epigenetic drug combination screening

CLASSIFICATION

Organism	Human
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This NCI-H1299 derivative (DKFZ #P-1045) contains a stably silenced EGFP reporter in the DAPK1 locus enabling epigenetic reactivation readout. G418-resistance cassette present. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

Morphology	Epithelial-like

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Cell type	Epithelial cells
Doubling time	approx. 24 to 36 hours

IDENTIFIERS

Citation

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NCBI TaxID	9606
Cellosaurus	CVCL_XB25

HANDLING AND CULTURE

Culture medium

RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO3 (Cytion article number 820700a)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 10% FBS

Dissociation reagent	Accutase

Subculturing

Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Split ratio	1 to 5
Seeding density	1 to 3 × 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.
To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Third-party agreements apply	Yes

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