Stable HEK293 Cells-Rpn11-HTBH

305719

GENERAL INFORMATION

Description

Stable HEK293 Cells-Rpn11-HTBH is a stably transfected derivative of the HEK293 (Human Embryonic Kidney 293) cell line, engineered to express a tagged version of Rpn11 (also known as PSMD14 or POH1), the deubiquitinase subunit of the 26S proteasome lid complex. Rpn11 is a Zn^{2+}-dependent JAMM-domain deubiquitinase that removes ubiquitin chains from proteasome-bound substrates during proteasomal degradation. The HTBH tag (hexahistidine-TEV-biotin acceptor peptide-hexahistidine) enables affinity purification of Rpn11-containing complexes under native conditions, making this line particularly suited for proteasome complex purification and interactome studies.
This cell line is applicable in studies of 26S proteasome biology, ubiquitin-proteasome pathway (UPS) regulation, Rpn11/PSMD14 function in protein quality control, proteasome assembly and dynamics, and proteasome inhibitor mechanism of action. It is also used for affinity purification of native proteasome complexes and as a model for studying deubiquitinase biology in the context of the proteasome. The HTBH tagging system enables highly stringent purification of biotinylated complexes using streptavidin-based pulldowns.

Tissue	Kidney
Disease	Transformed/immortalized fetal kidney (HEK293 background; Rpn11-HTBH transgene)

Applications

26S proteasome biology; Rpn11/PSMD14 function; ubiquitin-proteasome pathway; proteasome complex purification; deubiquitinase biology; HTBH-tag affinity purification; proteasome interactome studies

CLASSIFICATION

Organism	Human
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This HEK293 derivative contains a stably integrated Rpn11-HTBH expression cassette (hexahistidine-TEV-biotin acceptor peptide-hexahistidine tagged Rpn11/PSMD14). This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

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

IDENTIFIERS

Citation

Stable HEK293 Cells-Rpn11-HTBH (Cytion catalog number 305719)

NCBI TaxID	9606

Cytion GmbH
Nikola-Tesla-Str. 3
69124 Heidelberg
Tel: +49 (0)6221 405780
info@cytion.com
cytion.com

Registered office Heidelberg
VAT ID: DE283292917
Local court Mannheim - HRB 714394
CEO / Geschäftsführer: Jonathan Steubing

Banking information
Sparkasse Heidelberg
IBAN: DE35 6725 0020 0009 2076 86
BIC: SOLADES1HDB

HANDLING AND CULTURE

Culture medium

DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

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

Supplements

Supplement the medium with 10% FBS

Dissociation reagent	Most cells detach in PBS, if necessary add Accutase 5 min RT

Subculturing

Remove medium, wash with PBS without calcium and magnesium, cover with Accutase, incubate 8–10 min at RT, resuspend in medium, centrifuge 300×g 3 min, discard supernatant, reseed in fresh medium.

Split ratio	1 to 10
Seeding density	2 to 4 × 10^4 cells/cm^2
Fluid renewal	Every 2 to 3 days

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 200 x g for 5 minutes, carefully discard the supernatant containing freezing medium.
Follow the procedure described under Post-Thaw Recovery

QUALITY CONTROL AND CRYOPRESERVATION

Freeze medium	As a cryopreservation medium, we use complete growth medium + 10% DMSO for adequate post-thaw viability.

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.

Material Transfer Agreement required	Yes

Cytion GmbH
Nikola-Tesla-Str. 3
69124 Heidelberg
Tel: +49 (0)6221 405780
info@cytion.com
cytion.com

Registered office Heidelberg
VAT ID: DE283292917
Local court Mannheim - HRB 714394
CEO / Geschäftsführer: Jonathan Steubing

Banking information
Sparkasse Heidelberg
IBAN: DE35 6725 0020 0009 2076 86
BIC: SOLADES1HDB