Product sheet

HEK293-Rpn11-HTBH Cells | 305719

Informações gerais

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. Stable HEK293 Cells-Rpn11-HTBH are maintained as an adherent culture in DMEM supplemented with 10% FBS and the appropriate selection antibiotic to maintain transgene expression at 37°C in a humidified 5% CO_2 atmosphere. Cells are subcultured with Accutase at 80–90% confluency (split ratio 1:5 to 1:10). Medium renewed every 2–3 days.
Organism	Human
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

Características

Morphology	Epithelial-like
Cell type	Epithelial cells
Growth properties	Adherent

Dados regulatórios

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

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Biosafety level	1
NCBI_TaxID	9606
GMO Status	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.

Dados biomoleculares

Manuseio

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)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	Most cells detach in PBS, if necessary add Accutase 5 min RT
Doubling time	approx. 24 to 36 hours
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
Freeze medium	As a cryopreservation medium, we use complete growth medium + 10% DMSO for adequate post-thaw viability.

Thawing and Culturing Cells	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 2. 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. 3. 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. 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening. 5. 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. 6. Centrifuge the mixture at 200 x g for 5 minutes, carefully discard the supernatant containing freezing medium. 7. Follow the procedure described under Post-Thaw Recovery
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
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.
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.

Controle de Qualidade e Análise Molecular