Protea^{select}-HEK293_Bio-Rpn11 Cell Line

Cat. No. **66-5012-001**
Lot. No. **30349**

Quantity: 2-4 x 10^6 cells
Storage: cryopreserved

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

Background

The ubiquitin–proteasome system (UPS) targets selected proteins for degradation by the 26S proteasome. The initial steps in this pathway generate proteins that are covalently tagged with a polyubiquitin chain that is then recognized by ubiquitin receptors of the 26S proteasome. This is a large complex composed of a 20S catalytic core particle and two 19S regulatory particles (Kok *et al.*, 1993) that catalyse the final step in the pathway. While the 20S particle is composed of a catalytic chamber for protein degradation, collectively the proteins that comprise the 19S particle perform several proteasomal functions that include recognition of ubiquitylated substrates, cleavage of the polyubiquitin chain for ubiquitin recycling, control of access to the 20S proteolytic chamber, and substrate unfolding and subsequent translocation into the 20S core particle for degradation (Boehringer *et al.*, 2012). Mammalian proteasomes are associated with three DUBs: USP14, UCHL5 (UCH37) and Rpn11 (POH1). UCHL5 and USP14 reside on the regulatory particle and remove ubiquitin from the substrate before substrate degradation whereas Rpn11's activity is delayed until the proteasome is committed to degrading the substrate (Lee *et al.*, 2010). The DUB activity of USP14 is known to be activated by proteasomes.

To fully understand the function and regulation of the proteasome complex, an important step is to elucidate its subunit composition and post-translational modifications. Toward

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Culture Characteristics

Species: human

Source: embryonic kidney

Quantity: 2-4 x10^6 cells in 1 ml 50% complete media, 40% fetal bovine serum (FBS), 10% DMSO

Culture Medium: DMEM high glucose (HG), 10% FBS, 2 mM L-Glutamine, 1x pen/strep

To maintain selection of Rpn11 it is recommended to occasionally supplement the media with Puromycin at a final concentration of 2.5 µg/ml. Puromycin selection is recommended for at least 1 week, every 2 months or more.

Growth Mode: adherent

Passage Number: P11

Storage: cryopreserved

Culture Conditions: Thaw the cells by swirling the vial in a 37ºC water bath. Place 10 ml of culture media (without Puromycin) into a T75 flask and pipette cells into the flask containing the media. Place the T75 flask containing the cells into a 37ºC 5% CO_2 incubator overnight and replace with fresh media the next morning.

Recommended sub-culture routine: This cell line attaches loosely to the tissue culture flask thus the procedure is designed to ensure recovery of all cells. When the HEK293_Bio-Rpn11 cells reach confluency, collect and keep culture media from the T75 flask in a 50 ml tube and rinse cells with 10 ml PBS. Collect the PBS from the T75 flask (combine with media recovered above) and add 2 ml 0.05% Trypsin-EDTA (1x) to the flask. Incubate the flask of HEK293_Bio-Rpn11 cells in a 37°C 5% CO_2 incubator for a few minutes until the cells begin to detach, knock the side of the flask to fully detach the cells, neutralise with 10 ml of media and combine with the media and PBS recovered above. Centrifuge to pellet cells, discard the supernatant and resuspend in 5 ml media. Split 1:5 into new T75 flasks. Refeed the HEK293_Bio-Rpn11 cells every 3-4 days and passage as required.

Ubiquigent recommends not passaging past P30.

Description of Transgene: Please see page 2

Quality Assurance

Mycoplasma: Negative. The cells were previously positive for mycoplasma and have been treated using Plasmocin for 2 weeks with a final concentration of 25 µg/ml. Subsequently, they have been grown, re-tested and are now certified mycoplasma negative. Ubiquigent recommends, as for all cell lines, testing for mycoplasma status occasionally.

Morphology: Epithelial

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Lot-specific COA version tracker: v1.0.0

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Background

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this goal, Wang *et al.* (2007) have developed an affinity purification strategy using a derivative of the HB tag for rapid isolation of the human 26S proteasome complex for subsequent proteomic analysis. The purification of the complex is achieved from a stable HEK293 cell line expressing a HB-tagged (6His/Biotin-tagged) proteasome subunit (hRpn11) and by high-affinity streptavidin binding with TEV cleavage elution. The 'Biotin-tag' consists of a Biotin Acceptor Peptide sequence recognised and biotinylated by the enzyme BirA.

References:

Boehringer J, Riedinger C, Paraskevopoulos K, Johnson EO, Lowe ED, Khoudian C, *et al.* (2012) Structural and functional characterization of Rpn12 identifies residues required for Rpn10 proteasome incorporation. *Biochem J* **448**, 55-65.

Kok K, Hofstra R, Pilz A, van den Berg A, Terpstra P, Buys CH, *et al.* (1993) A gene in the chromosomal region 3p21 with greatly reduced expression in lung cancer is similar to the gene for ubiquitin-activating enzyme. *Proc Natl Acad Sci USA* **90**, 6071-6075.

Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, *et al.* (2010) Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* **467**, 179-184.

Wang X, Chen CF, Baker PR, Chen PL, Kaiser P and Huang L (2007) Mass spectrometric characterization of the affinity-purified human 26S proteasome complex. *Biochemistry* **46**, 3553-3565.

Culture Characteristics

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Description of Transgene: This stable cell line expresses hRpn11-HTBH (6His/TEV/Biotin Acceptor Peptide/6His-tagged).

hRpn11	*6xhis*	*TEV*	*Biotin Acceptor Peptide*	*6xhis*

Schematic representation of hRpn11 fused to the HTBH tag at its C-terminus. The HTBH tag consists of two hexahistidine tags, a TEV cleavage site, and a bacterially derived peptide sequence known as the Biotin Acceptor Peptide that is biotinylated *in vivo* by the enzyme BirA. The biotinylated hRpn11-HTBH protein inserts itself as a component of the proteasome complex enabling the multi-subunit complex to be purified using streptavidin capture protocols (Wang *et al.* 2007; Lee *et al.* 2010).

Applications

- Purifying proteasome complexes
- Proteasome subunit composition analysis (Wang *et al.* 2007)
- Proteasome catalytic assays (see also 26S Proteasome Cat. No. 65-1010-010 and 26S Proteasome [Ub-VS treated] Cat. No. 65-1020-010 products)
- Activating USP14 (Lee *et al.* 2010; see also USP14 Activation Kit Cat. No. 67-0014-001 and Activated USP14 DUB Assay Kit Cat. No. 67-0015-096 products)

Limited Use Licence (in respect of product Cat# 66-5012-001)

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