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14-535ACL: TLR6/HEK293 Stable Cell Line

Application : Functional Assay

Description

TLR6/HEK293 Stable Cell Line is a stably transfected HEK293 cell line which expresses human Toll-like receptor 6 (TLR6, also designated as CD286). TLR6 forms a heterodimer with TLR2, and then the TLR2-TLR6 dimer responds to multiple diacyl lipopeptides derived from Gram-positive bacteria and mycoplasma, which leads to the NF-kB-induced pro-inflammatory cytokine production and activation of innate immune responses. *Note that TLR6 in the TLR6/HEK293 stable cell line contains the N-terminal HA tag (Figure 1).*

Sequence data: Human TLR6 (accession number NP_001381482)

MTKDKEPIVKSFHFVCLMIIIVGTRIQFSDGNEFAVDKSKRGLI
HVPKDLPLKTKVLDMSQNYIAELQVSDMSFLSELTVLRLSHNRIQLLDLSVFKFNQDL
EYLDLSHNQLQKISCHPIVSFRHLDLSFNDFKALPICKEFGNLSQLNFLGLSAMKLQK
LDLLPIAHLHLSYILLDLRNYYIKENETESLQILNAKTLHLVFHPTSLFAIQVNISVN
TLGCLQLTNIKLNDDNCQVFIKFLSELTRGSTLLNFTLNHIETTWKCLVRVFQFLWPK
PVEYLNIYNLTIIESIREEDFTYSKTTLKALTIEHITNQVFLFSQTALYTVFSEMNIM
MLTISDTPFIHMLCPHAPSTFKFLNFTQNVFTDSIFEKCSTLVKLETLILQKNGLKDL
FKVGLMTKDMPSLEILDVSWNSLESGRHKENCTWVESIVVLNLSSNMLTDSVFRCLPP
RIKVLDLHSNKIKSVPKQVVKLEALQELNVAFNSLTDLPGCGSFSSLSVLIIDHNSVS
HPSADFFQSCQKMRSIKAGDNPFQCTCELREFVKNIDQVSSEVLEGWPDSYKCDYPES
YRGSPLKDFHMSELSCNITLLIVTIGATMLVLAVTVTSLCIYLDLPWYLRMVCQWTQT
RRRARNIPLEELQRNLQFHAFISYSEHDSAWVKSELVPYLEKEDIQICLHERNFVPGK
SIVENIINCIEKSYKSIFVLSPNFVQSEWCHYELYFAHHNLFHEGSNNLILILLEPIP
QNSIPNKYHKLKALMTQRTYLQWPKEKSKRGLFWANIRAAFNMKLTLVTENNDVKS

Product Info

Amount :	1 Vial
Content :	Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO
Storage condition :	Immediately upon receipt, store in liquid nitrogen.

Application Note

Application:.

- Functional assay.

Culture conditions:

Cells should be grown at 37°C with 5% CO_2 using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 10 µg/ml of Blasticidin.

It is recommended to quickly thaw the frozen cells upon receipt or from liquid nitrogen in a 37^{O}C water-bath, transfer

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to a tube containing 10 ml of growth medium without Blasticidin, spin down cells, resuspend cells in pre-warmed growth medium without Blasticidin, transfer resuspended cells to T25 flask and culture in 37^{0}C-CO_2 incubator.

Leave the T25 flask in the incubator for 1~2 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are over 90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Blasticidin. Cells should be split before they reach complete confluence.

To passage the cells, detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cells suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.

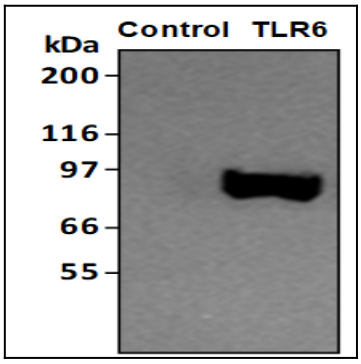

Fig-1: Western blot analysis of TLR6 expression in the TLR6/HEK293 cell line. Cell lysates were analyzed by SDS-PAGE followed by Western blotting using anti-HA antibody. Note that TLR6 in the TLR6/HEK293 stable cell line contains the N-terminal HA tag. Control, parental HEK293 cell lysate; TLR6, TLR6/HEK293 cell lysate.