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14-531ACL: TLR2/HEK293 Stable Cell Line

Application : Functional Assay

Description

TLR2/HEK293 Stable Cell Line is a stably transfected HEK293 cell line which expresses human Toll-like receptor 2 (TLR2, also designated as CD282). TLR2 mediates immune responses by recognition of lipid-containing pathogen-associated molecular patterns (PAMPs) such as lipoteichoic acid and di- and tri-acylated cysteine-containing lipoproteins. TLR2 forms heterodimers with TLR1 or TLR6 as the initial step in a signal transduction cascade which leads to significant innate immune responses as well as development of adaptive immunity. TLR2-TLR1 heterodimers recognize triacylated lipopeptides from Gram-negative bacteria and mycoplasma, while TLR2-TLR6 heterodimers recognize diacylated lipopeptides from Gram-positive bacteria and mycoplasma. *Note that TLR2 in the TLR2/HEK293 stable cell line contains the N-terminal HA tag (Figure 2), which does not interfere with TLR2 activity as confirmed by functional assay (Figure 1).*

Sequence data: Human TLR2 (accession number NP_001305716)

MPHTLWMVWVLGVIISLSKEESSNQASLSCDRNGICKGSSGSLN
SIPSGLTEAVKSLDLSNNRITYISNSDLQRCVNLQALVLTSNGINTIEEDSFSSLGSL
EHLDLSYNYLSNLSSSWFKPLSSLTFLNLLGNPYKTLGETSLFSHLTKLQILRVGNMD
TFTKIQRKDFAGLTFLEELEIDASDLQSYEPKSLKSIQNVSHLILHMKQHILLLEIFV
DVTSSVECLELRDTDLDTFHFSELSTGETNSLIKKFTFRNVKITDESLFQVMKLLNQI
SGLLELEFDDCTLNGVGNFRASDNDRVIDPGKVETLTIRRLHIPRFYLFYDLSTLYSL
TERVKRITVENSKVFLVPCLLSQHLKSLEYLDLSENLMVEEYLKNSACEDAWPSLQTL
ILRQNHLASLEKTGETLLTLKNLTNIDISKNSFHSMPETCQWPEKMKYLNLSSTRIHS
VTGCIPKTLEILDVSNNNLNLFSLNLPQLKELYISRNKLMTLPDASLLPMLLVLKISR
NAITTFSKEQLDSFHTLKTLEAGGNNFICSCEFLSFTQEQQALAKVLIDWPANYLCDS
PSHVRGQQVQDVRLSVSECHRTALVSGMCCALFLLILLTGVLCHRFHGLWYMKMMWAW
LQAKRKPRKAPSRNICYDAFVSYSERDAYWVENLMVQELENFNPPFKLCLHKRDFIPG
KWIIDNIIDSIEKSHKTVFVLSENFVKSEWCKYELDFSHFRLFDENNDAAILILLEPI
EKKAIPQRFCKLRKIMNTKTYLEWPMDEAQREGFWVNLRAAIKS

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.

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

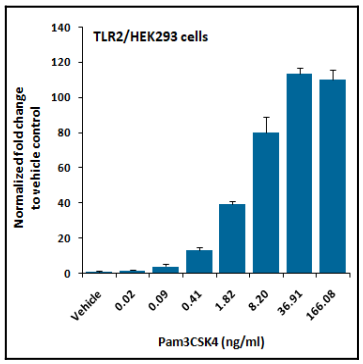

Fig-1: Functional analysis of the TLR2/HEK293 cell line. TLR2/HEK293 cells as well as parental HEK293 cells were transfected with NF-kB/Renilla luciferase reporter plasmid for 16 h. Cells were stimulated with different doses of Pam3CSK4 (Abeomics, Cat. #15-1011) for 16 h followed by luciferase assay using Luciferase Assay Reagent (Abeomics, Cat. #17-1101).

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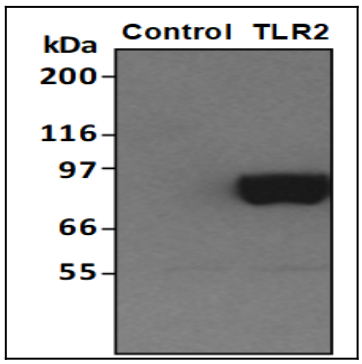

Fig-2: Western blot analysis of TLR2 expression in the TLR2/HEK293 cell line. Cell lysates were analyzed by SDS-PAGE followed by Western blotting using anti-HA antibody. Note that TLR2 in the TLR2/HEK293 stable cell line contains the N-terminal HA tag. Control, parental HEK293 cell lysate; TLR2, TLR2/HEK293 cell lysate.