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14-533ACL: TLR4/HEK293 Stable Cell Line

Application : Functional Assay

Description

TLR4/HEK293 Stable Cell Line is a stably transfected HEK293 cell line which expresses human Toll-like receptor 4 (TLR4, also designated as CD284). TLR4 mediates immune responses by recognition of lipoplysaccharides (LPS) with the help of LPS-binding protein, CD14 and MD-2. TLR4 stimulation uniquely triggers two signaling pathways named the MyD88-dependent pathway and the TRIF-dependent pathway, which lead to the production of two sets of cytokines such as proinflammatory cytokines and type I interferons/chemokines, respectively. *Note that TLR4 in the TLR4/HEK293 stable cell line contains the N-terminal HA tag (Figure 2), which does not interfere with TLR4 activity as confirmed by functional assay (Figure 1).*

Sequence data: Human TLR4 (accession number NP_612564)

MMSASRLAGTLIPAMAFLSCVRPESWEPCVEVVPNITYQCMELN
FYKIPDNLPFSTKNLDLSFNPLRHLGSYSFFSFPELQVLDLSRCEIQTIEDGAYQSLS
HLSTLILTGNPIQSLALGAFSGLSSLQKLVAVETNLASLENFPIGHLKTLKELNVAHN
LIQSFKLPEYFSNLTNLEHLDLSSNKIQSIYCTDLRVLHQMPLLNLSLDLSLNPMNFI
QPGAFKEIRLHKLTLRNNFDSLNVMKTCIQGLAGLEVHRLVLGEFRNEGNLEKFDKSA
LEGLCNLTIEEFRLAYLDYYLDDIIDLFNCLTNVSSFSLVSVTIERVKDFSYNFGWQH
LELVNCKFGQFPTLKLKSLKRLTFTSNKGGNAFSEVDLPSLEFLDLSRNGLSFKGCCS
QSDFGTTSLKYLDLSFNGVITMSSNFLGLEQLEHLDFQHSNLKQMSEFSVFLSLRNLI
YLDISHTHTRVAFNGIFNGLSSLEVLKMAGNSFQENFLPDIFTELRNLTFLDLSQCQL
EQLSPTAFNSLSSLQVLNMSHNNFFSLDTFPYKCLNSLQVLDYSLNHIMTSKKQELQH
FPSSLAFLNLTQNDFACTCEHQSFLQWIKDQRQLLVEVERMECATPSDKQGMPVLSLN
ITCQMNKTIIGVSVLSVLVVSVVAVLVYKFYFHLMLLAGCIKYGRGENIYDAFVIYSS
QDEDWVRNELVKNLEEGVPPFQLCLHYRDFIPGVAIAANIIHEGFHKSRKVIVVVSQH
FIQSRWCIFEYEIAQTWQFLSSRAGIIFIVLQKVEKTLLRQQVELYRLLSRNTYLEWE
DSVLGRHIFWRRLRKALLDGKSWNPEGTVGTGCNWQEATSI

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.

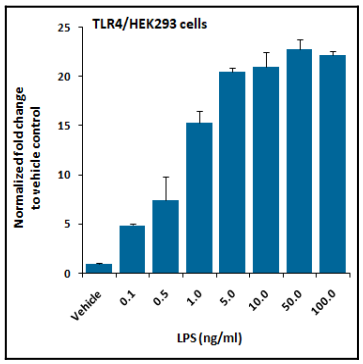

Fig-1: Functional analysis of the TLR4/HEK293 cell line. TLR4/HEK293 cells as well as parental HEK293 cells were transfected with CD14, MD-2 and NF-kB/Renilla luciferase reporter plasmids for 16 h. Cells were stimulated with different doses of LPS (Abeomics, Cat. #15-1013) for 16 h followed by luciferase assay using Luciferase Assay Reagent (Abeomics, Cat. #17-1101).

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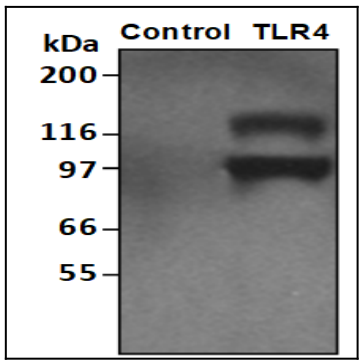

Fig-2: Western blot analysis of TLR4 expression in the TLR4/HEK293 cell line. Cell lysates were analyzed by SDS-PAGE followed by Western blotting using anti-HA antibody. Note that TLR4 in the TLR4/HEK293 stable cell line contains the N-terminal HA tag. Control, parental HEK293 cell lysate; TLR4, TLR4/HEK293 cell lysate.