

14-534ACL: TLR5/HEK293 Stable Cell Line

Application : Functional Assay

Description

TLR5/HEK293 Stable Cell Line is a stably transfected HEK293 cell line which expresses human Toll-like receptor 5 (TLR5, also designated as CD285). TLR5 mediates immune responses by recognition of bacterial flagellin, of which activation involves in various human diseases including inflammatory bowel disease, rheumatoid arthritis, osteoclastogenesis, gastric cancer, cervical cancer and ovarian cancer. *Note that TLR5 in the TLR5/HEK293 stable cell line contains the N-terminal HA tag (Figure 2), which does not interfere with TLR5 activity as confirmed by functional assay (Figure 1).*

Sequence data: Human TLR5 (accession number NP_003259)

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MGDHLDLLLGVVLMAGPVFGIPSCSFDGRIAFYRFCNLTQVPQV
LNTTERLLLSFNIRTVTASSFPFLEQLQLELGSQYPTLTIDKEAFRNLPNLRILD
GSSKIYFLHPDAFQGLFHLFELRLYFCGLSDAVLKDGYFRNLKALTRLDLSKNQIRSL
YLHPSFGKLNLSKSIDFSSNQIFLVCEHELEPLQGKTLSSFLAANSLSRVSVSDWGK
CMNPFRRNMVLEILDVSGNGWTVDTGNFSNAISKSQAFSLILAHHIMGAGFGFHNIKD
PDQNTFAGLARSSVRHLDLSHGFFVSLNSRVFETLKDVLNLAYNKINKIADEAFYG
LDNLQVLNLSYNLLGELYSSNFYGLPKVAYIDLQKNHIAIQDQTFKLEKLQTLDLR
DNALTTIHFIPIPIFLSGNKLVTLPKINLTANLIHLSNRLENLDILYFLLRVPHL
QILILNQNRFFSSCGDQTPSENPSLEQLFGENMLQLAWETELCWDVFEGLSHLQVLY
LNHNYLNSLPPGVFSLTALRGLSLNSNRRLTVLSHNDLPANLEILDISRNQLLAPNPD
VFVLSVLDTHTNKFICECELSFTINWLNHTNVTIAGPPADIYCVYPDSFSGVSLFSL
STEGCDEEEVLKSLKFSLVFVCTVTLTFLMTILTVTKFRGFCFICYKTAQRLVFKDH
PQGTEPDMYKYDAYLCFSSKDFTWVQNALLKHLDQYSDQNRFNLCFEERDFVPGENR
IANIQDAIWNSRKIVCLVSRHFLRDGWCLEAFSYAQGRCLSDNLNLSALIMVVVGSLSQY
QLMKHQSIRGFVQKQYLRWPEDLQDVGWFLHKLSSQILKKEKEKKDNNIPLQTVAT
IS
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Product Info

Amount : 1 Vial
Content : Each vial contains $2 \sim 3 \times 10^6$ 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% CO₂ 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°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°C-CO₂ 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 ratio = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.

LIMITED USE RESTRICTIONS:

THIS PRODUCT IS SOLELY FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

By use of this product, user agrees to be bound by the terms of this limited use statement.

This product is solely for Internal Research Purposes and not for Commercial Purposes. Commercial Purposes include, but are not limited to (1) use of the cell line in manufacturing; (2) use of the cell line to provide a service, information or data; (3) use of the cell line for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the cell line whether or not such cell lines are resold for use in research. The buyer cannot sell, give or otherwise transfer this product to a third party.

Commercial License Agreement is available for non-research use if applicable. Please contact Abeomics (info@abeomics.com).

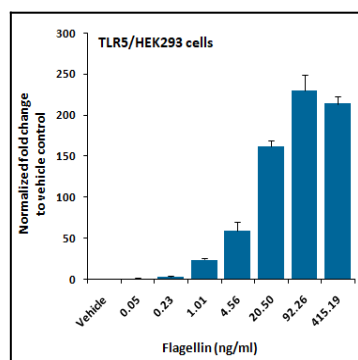


Fig-1: Functional analysis of the TLR5/HEK293 cell line. TLR5/HEK293 cells as well as parental HEK293 cells were transfected with NF-κB/Renilla luciferase reporter plasmid for 16 h. Cells were stimulated with different doses of flagellin (Abeomics, Cat. #15-1014) for 16 h followed by luciferase assay using Luciferase Assay Reagent (Abeomics, Cat. #17-1101).

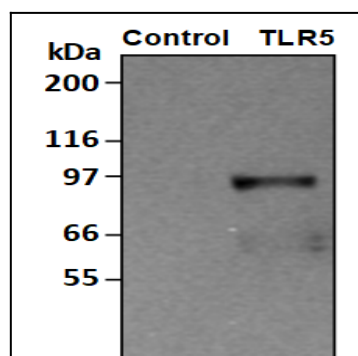


Fig-2: Western blot analysis of TLR5 expression in the TLR5/HEK293 cell line. Cell lysates were analyzed by SDS-PAGE followed by Western blotting using anti-HA antibody. Note that TLR5 in the TLR5/HEK293 stable cell line contains the N-terminal HA tag. Control, parental HEK293 cell lysate; TLR5, TLR5/HEK293 cell lysate.