

14-524ACL: ACE2/HEK293 Stable Cell Line

Application : Functional Assay

Description

ACE2/HEK293 Stable Cell Line is a stably transfected HEK293 cell line which expresses human angiotensin-converting enzyme 2 (ACE2). ACE2 is a type I transmembrane metalloenzyme located on the outer surface of endothelial cells in the lung, arteries, heart, kidney and intestines. ACE2 cleaves the carboxyl-terminal amino acid phenylalanine from angiotensin II and hydrolyses it into the vasodilator angiotensin. ACE2 also serves as an entry receptor for some coronaviruses including HCoV-NL63, SARS-CoV and SARS-CoV-2 as the virus that causes COVID-19.

Sequence data: hACE2 (accession number NP_068576)

```
MSSSSWLLLSLVAVTAAQSTIEEQAKTFLDKFNHEAEDLFYQSS
LASWNYNTNITEENVQNMNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQ
NGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNE
RLWAWESWRSEVGKQLRPLYEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYS
RGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWT
NLYSLTVPFGQKPNIDVTDAMVDQAWDAQRFKEAEKFFVSVGLPNMTQGFWENSMLT
DPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAAHEMGGHIQYDMAYAAQPFL
RNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTL
PFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVPEVPHDETYCDPASLFHVS
DYSFIRYYTRTLYQFQFQALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPW
TLAENVVGAKNMNVRPLNLYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISL
KSALGDKAYEWNDEMILFRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRISFN
FFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPNQPPVS
IWLIVFGVVMGVVVGIVLIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNT
DDVQTSF
```

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:

- Screen for antibodies of human ACE2 through Flow Cytometry.
- Protein binding study.

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 250 µg/ml of Hygromycin.

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 Hygromycin, spin down cells, resuspend cells in pre-warmed growth medium without Hygromycin, 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 Hygromycin. 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).

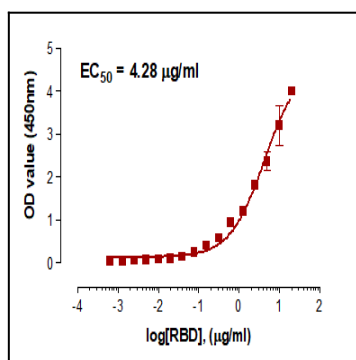


Fig-1: Binding of biotinylated SARS-Cov-2 Spike RBD protein to human ACE2 in the ACE2/HEK293 stable cell line. ACE2/HEK293 cells were probed with different amounts of biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) and analyzed through In-Cell ELISA using HRP-Streptavidin for detection.

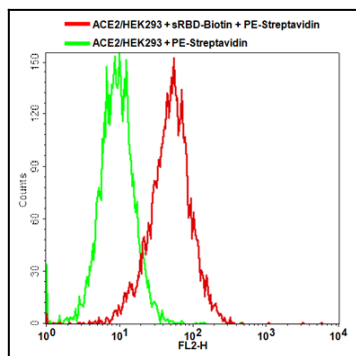


Fig-2: Detection of ACE2 expression in ACE2/HEK293 cells by flow cytometry. ACE2/HEK293 cells were probed with biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) at 1×10^5 cells per $1 \mu\text{g}$ protein on ice for 2 hours. Cells were washed with flow buffer and then incubated with PE-labeled Streptavidin on ice for 20 min. Cell were washed and then analyzed by flow cytometry.

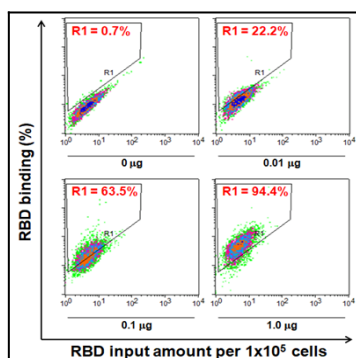


Fig-3: Binding of biotinylated SARS-Cov-2 Spike RBD protein to human ACE2 in the ACE2/HEK293 stable cell line. ACE2/HEK293 cells were probed with different amounts of biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) and analyzed by flow cytometry through fluorescent-labeled Streptavidin detection.

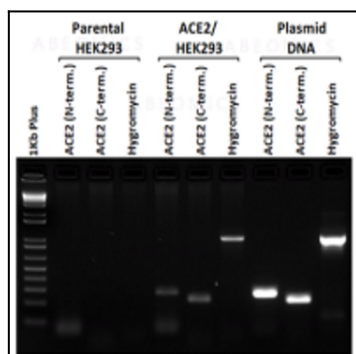


Fig-4: Genomic PCR analysis. Genomic DNAs were purified from ACE2/HEK293 stable cells and parental HEK293 cells, and used as the PCR templates. PCR was performed using the target gene construct-specific primer sets, and the plasmid DNA of target gene construct was used as positive control.

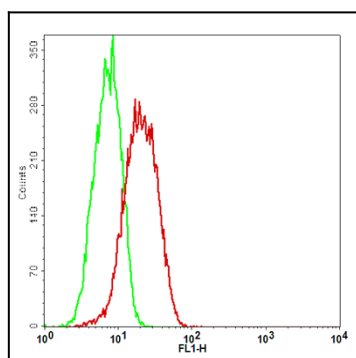


Fig-5: Cell Surface staining of human ACE2 in the ACE2/HEK293 stable cell line. ATTO 488-conjugated anti-hACE2 antibody (clone AC18F; Abeomics, Cat. No.: 10-10031-AT488) was used at $1 \mu\text{g}/1 \times 10^6$ cells (Red). ATTO 488-conjugated mouse IgG1 was used as isotype control at $1 \mu\text{g}/1 \times 10^6$ cells (Green).