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14-525ACL: ACE2/VERO Stable Cell Line

Application : Functional Assay

Description

ACE2/VERO Stable Cell Line is a stably transfected Vero E6 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
LASWNYNTNITEENVQNMNNAGDKWSAFLKEQSTLAQMYPLQEIQNLTVKLQLQALQQ
NGSSVLSEDKSKRLNTILNTMSTIYSTGKVCNPDNPQECLLLEPGLNEIMANSLDYNE
RLWAWESWRSEVGKQLRPLYEEYVVLKNEMARANHYEDYGDYWRGDYEVNGVDGYDYS
RGQLIEDVEHTFEEIKPLYEHLHAYVRAKLMNAYPSYISPIGCLPAHLLGDMWGRFWT
NLYSLTVPFGQKPNIDVTDAMVDQAWDAQRIFKEAEKFFVSVGLPNMTQGFWENSMLT
DPGNVQKAVCHPTAWDLGKGDFRILMCTKVTMDDFLTAHHEMGHIQYDMAYAAQPFLL
RNGANEGFHEAVGEIMSLSAATPKHLKSIGLLSPDFQEDNETEINFLLKQALTIVGTL
PFTYMLEKWRWMVFKGEIPKDQWMKKWWEMKREIVGVVEPVPHDETYCDPASLFHVSN
DYSFIRYYTRTLYQFQFQEALCQAAKHEGPLHKCDISNSTEAGQKLFNMLRLGKSEPW
TLALENVVGAKNMNVRPLLNYFEPLFTWLKDQNKNSFVGWSTDWSPYADQSIKVRISL
KSALGDKAYEWNDNEMYLFRSSVAYAMRQYFLKVKNQMILFGEEDVRVANLKPRISFN
FFVTAPKNVSDIIPRTEVEKAIRMSRSRINDAFRLNDNSLEFLGIQPTLGPPNQPPVS
IWLIVFGVVMGVIVVGIVILIFTGIRDRKKKNKARSGENPYASIDISKGENNPGFQNT
DDVQTSF

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:.

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

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 250 µg/ml of Hygromycin.

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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 Hygromycin, spin down cells, resuspend cells in pre-warmed growth medium without Hygromycin, 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 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 ration = 1:10 to 1:20 weekly. To achieve satisfactory results, cells should not be passaged over 16 times.

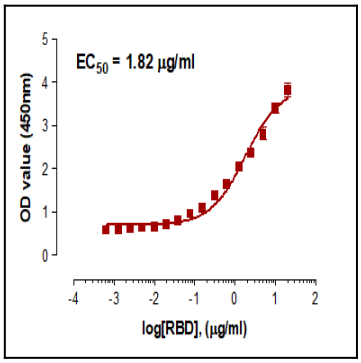

Fig-1: Binding of biotinylated SARS-Cov-2 Spike RBD protein to human ACE2 in the ACE2/VERO stable cell line. ACE2/VERO cells were probed with different amounts of biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) and analyzed through In-Cell ELISA using HRP-Streptavidin for detection.

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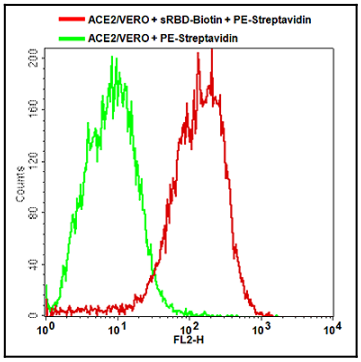

Fig-2: Detection of ACE2 expression in ACE2/VERO cells by flow cytometry. ACE2/VERO cells were probed with biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) at 1 x 10^5 cells per 1 µg protein on ice for 2 hours. Cells were washed with flow buffer and then incubated with PE-labeled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

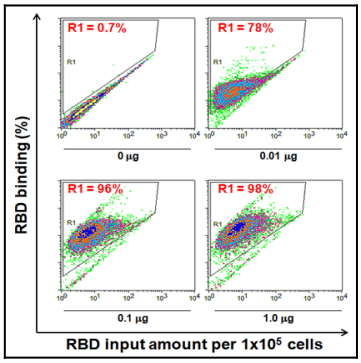

Fig-3: Binding of biotinylated SARS-Cov-2 Spike RBD protein to human ACE2 in the ACE2/VERO stable cell line. ACE2/VERO cells were probed with different amounts of biotinylated SARS-Cov-2 Spike RBD protein (Abeomics, Cat. No. 21-1005-B) and analyzed by flow cytometry through fluorescent-labeled Streptavidin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

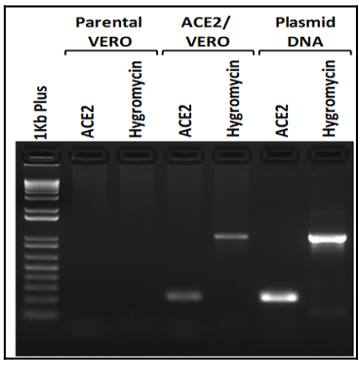

Fig-4: Genomic PCR analysis. Genomic DNAs were purified from ACE2/VERO stable cells and parental Vero E6 cells, and used as the PCR templates. PCR was performed using the target gene construct-specific primer sets, and the plasmid DNA of target gene construct was used as positive control.

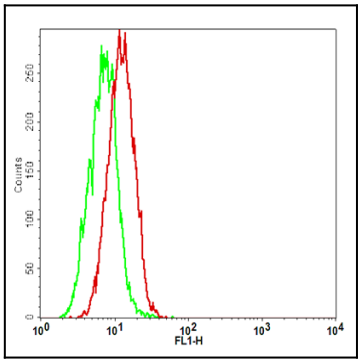

Fig-5: Cell Surface staining of human ACE2 in the ACE2/VERO stable cell line. ATTO 488-conjugated anti-hACE2 antibody (clone AC18F; Abeomics, Cat. No.: 10-10031-AT488) was used at 1ug/ 1x10^6 cells (Red). ATTO 488-conjugated mouse IgG1 was used as isotype control at 1µg/ 1x10^6 cells (Green).