APX273Ge02 100µg Active Spike Protein (SP) Organism Species: *Pan-species (General) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Met1~Arg685 Tags: N-terminal His Tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.01% skl and 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.0 Predicted Molecular Mass: 60.1kDa Accurate Molecular Mass: 60kDa as determined by SDS-PAGE reducing conditions. [USAGE]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDG VYFASTEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMD LEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYV GYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRK RISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKV GGNYNYLYRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLV KNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVA IHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRAR

[ACTIVITY]

The coronavirus spike (S) glycoprotein is a class I viral fusion protein on the outer envelope of the virion that plays a critical role in viral infection by recognizing host cell receptors and mediating fusion of the viral and cellular membranes. The S proteins consist of two subunits, S1 and S2.Besides,S1 binding to ACE2 and CLEC4M/DC-SIGNR receptors and internalizationof thevirus into the endosomes of the host cell induces conformational changes in the S glycoprotein. Thus a binding ELISA assay was conducted to detect the interaction of recombinant human ACE2, mouse ACE, rat ACE2 and recombinant Spike glycoprotein(S1). Briefly, ACE were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100µl were then transferred to S1-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ACE2 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37°C. Finally, add 50µl stop solution to the wells and read at 450nm immediately. The binding activity of ACE2 and S1 was shown in Figure 1, and this effect was in a dose dependent manner.

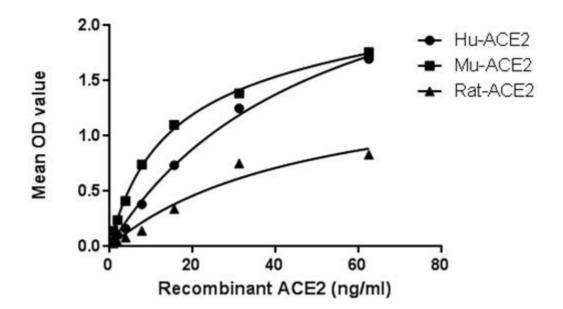
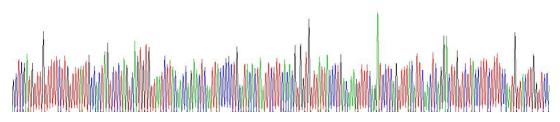


Figure 1. The binding activity of ACE2 with S1

[IDENTIFICATION]



QCXX/ACTMITCTTIC/CCGIGGIGTTATI/CCCIG/CM/GTTIC/CAICCIC/GTTIC/CTIC/ACIC/CG/CTIGTICTACCTTCTTIC/AAIGT/CTICGTIC/

Figure 2. Gene Sequencing (extract)

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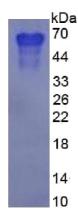


Figure 3. SDS-PAGE

Sample: Active recombinant SP, General

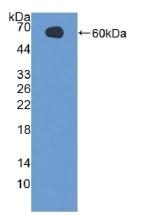


Figure 4. Western Blot

Sample: Recombinant SP, General;

Antibody: Rabbit Anti-General SP Ab (PAX273Ge02)

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.

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