3th Edition, revised in August, 2020

（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

COVID-19-pseudovirus for PCR 1.0

Catalog No： E-PV-0011

Size： 1mL

Cat.	Products	1 mL	Storage
E-PV-0011	COVID-19-pseudovirus for PCR 1.0	1 mL	-80°C
Manual		**One Copy**	

1）Target value (log value) ≥ 5×10^{6} copies/ml。

2）This product can be directly used as a template. This product simulates the viral structure of nCOV, and can also be used as a template after the RNA is extracted as required. The specific extraction method is shown in the Manual.

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Introduction

This product constructed a cell line to produce the ORF1ab and N gene mRNAs of COVID-19 virus stably (see appendix for detailed sequence), and on the basis of this cell line, the preparation of pseudoviruses was completed in the way of lentivirus packaging. This product can be used to verify the ability of COVID-19 virus nucleic acid detection reagent and the performance of the extraction kit. It can be used to carry out testing ability verification and laboratory quality control for COVID-19 virus nucleic acid detection laboratory.

This product is a pseudovirus obtained by building stable cell lines without plasmid residue or DNA residue. It can be used for ab quality control of the COVID-19 virus nucleic acid detection kit-the whole detection process from RNA extraction to reverse transcription to PCR amplification.

Features

1) The pseudovirus has no pathogenicity and only requires a level P2 laboratory, which can be applied to the detection limit of detection kits in the field of scientific research.

2) This product is a fake virus obtained by building stable cell lines without plasmid or DNA residues. It can be used for ab quality control of the novel Coronavirus nucleic acid detection kit.

Product Details

Experimental Procedure

- **Fake virus melt**

  Remove the pseudovirus from the -80°C refrigerator, melt it in an ice bath or naturally melted at 4°C, and then take the relevant experimental operation.

- **RNA extraction of pseudovirus(extraction example)**

  1. Take 200 μL of this product mixed with 30 μL of other irrelevant RNA (10~100 ng/μL) and then add 800 μL RNAiso Plus. Blow and mix with a pipette, and incubate at RT for 5 min.

  2. Add 160 μL chloroform to step 1 above and mix it upside down until the solution becomes milky white. Incubate at RT for 5 min. Centrifuge at 12000 g for 15 min and take it out carefully. At this time, the homogenate is divided into three layers-colorless supernatant (containing RNA), intermediate white protein (mostly DNA), and colored underlying organic phase.

  3. Take the supernatant (about 700 μL) and transferred to a new centrifuge tube. Add 500 μL isopropanol into the tube, mix it upside down and incubate for 20~30 min at RT.

  4. Centrifuge at 12000 g for 10 min. Remove the supernatant carefully. Add 800 μL 75% ethanol, wash upside down and centrifuge at 12,000 g for 10 min.

  5. Remove the supernatant, dry for 5~10 min at RT with an ultra-clean air dryer, then add 30~50 μL RNase free water to dissolve the sample.

  6. (Optional) Add DNase I to treat the sample according to the DNase I manual.

- **qRT-PCR detection**

  According to the instructions of the qRT-PCR kit, relevant experimental operations can be performed.

Storage

Store at -80°C. To avoid repeated freezing-thawing, it is recommended to store at -80°C in separate packing.

Appendix

The virus sequence contained in this product

AGTTGACTTCGCAGTGGCTAACTAACATCTTTGGCACTGTTTATGAAAAACTCAAACCCGTCCTTGATTGGCTT
GAAGAGAAGTTTAAGGAAGGTGTAGAGACCCTGTGGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCG
GTATGTGGAAAGGTTATGGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAATC
GTTTTTACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTG
CTTTGCTGCTGCTTGACAGATTGAACC AGCTTGAGAGCAAAATGTCTGGTAAA

Primer and probe sequences are recommended (5'-3')

Primer/Probe	Base sequence(5'-3')
ORF1ab-F	CCC TGT GGG TTT TAC ACT TAA
ORF1ab-R	ACG ATT GTG CAT CAG CTG A
ORF1ab-Probe	CCG TCT GCG GTA TGT GGA AAG GTT ATG G
N-F	GGG GAA CTT CTC CTG CTA GAA T
N-R	CAG ACA TTT TGC TCT CAA GCT G
N-Probe	TTG CTG CTG CTT GAC AGA TT

Cautions

1) RNase free reagent and consumables should be used in the experiment.

2) Freezing and thawing will reduce the stability of the pseudovirus, thus affecting the nucleic acid extraction effect and PCR detection results. Repeated freezing and thawing should be avoided in use.

3) This product is not the full sequence of COVID-19 virus, please carefully confirm whether the selected primer probe is in the coverage area of this product before use. See appendix for virus sequence contained in this product.

4) Virus inactivation treatment may cause RNA degradation, so please choose this step reasonably based on the actual situation.

5) If it is necessary to dilute the product, PBS, normal saline or RNase Free Water can be used as dilution buffer.