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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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SARS-CoV-2 Nucleocapsid Protein ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of SARS CoV-2 Nucleocapsid protein in cell culture medium, nasal swab and saliva.

Catalogue Number LDG00016E

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO



96 wells



Store at 2-8℃

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Leadgene® SARS-CoV-2 Nucleocapsid Protein ELISA Kit

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the genus *Betacoronavirus*, family *Coronaviridae* that causes coronavirus disease 2019 (COVID-19). SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus. Like other coronaviruses, SARS-CoV-2 has four structural proteins: spike, envelope, membrane and nucleocapsid. The nucleocapsid protein (NP) is the most abundant protein in coronavirus that encapsulates the genomic RNA and often used as a marker in diagnostic assays due to its high immunogenicity.

Leadgene® SARS CoV-2 Nucleocapsid Protein ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of SARS-CoV-2 NP level in cell culture medium, nasal swab and saliva. The SARS-CoV-2 Nucleocapsid Protein ELISA kit is for research use only (RUO). Not suitable for use in therapeutic procedures.

2. Test principle

Leadgene® SARS CoV-2 Nucleocapsid Protein ELISA kit is used to detect SARS CoV-2 NP in samples by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-SARS CoV-2 NP monoclonal antibody to the solid phase. SARS CoV-2 NP in the samples conjugates on solid phase and then react with the HRP conjugated mouse anti-SARS CoV-2 NP monoclonal antibody. Subsequent wash steps will remove unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of SARS CoV-2 NP level is accomplished by comparing the absorbance value with standard curve.

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3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
SARS-CoV-2 nucleocapsid protein ELISA plate Stripwell microplate with 96 anti-SARS-CoV-2 NP monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard SARS-CoV-2 NP lyophilized from buffered protein solution with preservatives	2 vials (lyophilized form)	**Refer to the vial label for reconstitution volume.** Reconstitute by adding **Standard reconstitution buffer** to be a stock solution of 20 ng/mL. (see procedure, section 9.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	1 vial (2 mL)	**Ready** for use
Standard & Sample diluent buffer Buffered protein solution with preservatives	1 vial (20 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti- SARS-CoV-2 NP monoclonal antibody in buffered protein solution with preservatives	1 vial (60 μL)	**Dilute** 200 x with **HRP-antibody conjugated diluent buffer** (see reagent preparation, section 5.A)
HRP-antibody conjugated diluent buffer Buffered solution with preservatives	1 vial (12 mL)	**Ready** for use
10 X wash buffer 10-fold concentrated solution of buffered surfactant with preservatives	1 vial (22 mL)	**Dilute** 10 x with distilled water (see reagent preparation, section 5.B)
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	**Ready** for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	**Ready** for use
Microplate sealing film	1 sheet	N/A

4. Materials required but not provided

(1) High-quality distilled water

(2) 10 mL graduated pipettes

(3) 5 μL to 1000 μL adjustable single-channel micropipettes with disposable tips

(4) 50 μL to 300 μL adjustable multi-channel micropipettes with disposable tips

(5) Multi-channel micropipette reservoir

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(6) Disposable microcentrifuge tubes
(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) Timer
(9) Magnetic stirrer
(10) Vortex mixer
(11) Washer for microplates
(12) Stripwell microplate spectrophotometer capable of reading at 450 nm
(13) Clean paper towels
(14) Disposable gloves
(15) Discard container for bio-medical waste

5. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

A. **Working HRP-antibody conjugate (1 X):** Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **HRP-antibody conjugated diluent buffer** and homogenize by vortex.
B. **Working wash buffer (1 X):** Dilute 1 volume of **10 X wash buffer** with 9 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Storage and expiration date of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8℃ after used, except the reconstituted standard, it must be stored at -80℃.
- Avoid multiple freeze-thaw cycles of the frozen reconstituted standard, and if stored properly at -80℃, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8℃ in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

7. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains H_2SO_4) or saliva/swab specimens contacting with skin or eyes. If contacted, wash immediately and thoroughly with plenty of running and

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clean water.

- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Bring all the reagents and specimens to 15-30℃ prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Specimen collection, processing & storage

Cell culture supernatant, nasal swab and saliva are suitable for use in this assay. Sample stability has not been evaluated.

- Cell culture supernatant:

(1) Remove particles by centrifugation. Sample should be assayed immediately or store at -80°C. Avoid repeated freeze-thaw cycles.

- Nasal swab:

(1) Gently insert a sterile swab into the nostril of the patient, reaching the surface of the posterior nasopharynx.
(2) Swab over the surface of the posterior nasopharynx.
(3) Withdraw the sterile swab from the nasal cavity.
(4) Place the swab specimen in a clean, sterile centrifuge tube with Standard & Sample diluent buffer (about 300 µL). Rotate the swab for about 10 seconds and pressing the head against the inside of the tube to release the antigen in the swab.
(5) Remove the swab while squeezing the swab head against inside of the centrifuge tube for removing as much liquid as possible from the swab. Discard the swab in accordance with national biohazard waste disposal protocol.
(6) Extraction sample should be assayed immediately.

- Saliva:

(1) Collect saliva using centrifugation tube and centrifuge at 10,000 x g for 5 min. Sample (aqueous layer) should be assayed immediately.

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9. Procedure

(1) Evaluate the number of stripwell required to test the samples. Put the stripwells at room temperature (18-25℃) before use. The unused strips should be resealed in the bag and stored at 2-8℃. Each standard, blank, and sample should be assayed in duplicate.

(2) Standard and sample preparation:

- **Standard preparation (in microcentrifuge tubes):**

- **Refer to the vial label for the reconstitution volume.** Reconstitute the lyophilized standard with Standard Reconstitution Buffer to a final concentration of 20 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C.
- Add 300 µL Standard & Sample diluent buffer to 300 µL of 20 ng/mL standard to make a 10 ng/mL standard (Tube 1).
- Adding 300 µL of Standard & Sample diluent buffer to 300 µL of 10 ng/mL standard to make a 5 ng/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only containing Standard & Sample diluent buffer.

- **Sample preparation:**

- 100 µL Sample. If the initial assay found samples contain SARS-CoV-2 NP higher than the highest standard, the samples can be diluted with Standard & Sample diluent buffer and then re-assay the samples.

Add 100 µL of standards, blanks or samples into SARS CoV-2 Nucleocapsid Protein ELISA stripwell microplates (see Table 1), then add 100 µL of Working HRP-antibody conjugate (1 X) into each well. Cover with microplate sealing film and incubate sealed plate at RT for 1 hour in the dark.

(3) Remove the sealing film, aspirate the liquid from each well and then wash the plate six times with 300 µL Working wash buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.

(4) Add 100 µL of TMB into each well. Incubate for 15 minutes at RT in the dark.

(5) Add 50 µL Stop solution into each well.

(6) Read the absorbencies immediately at 450 nm after the Stop solution is added.

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Table 1 An example of orientation of standards, blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (10 ng/mL)	Standard 1 (10 ng/mL)	Sample 1	Sample 5
B	Standard 2 (5 ng/mL)	Standard 2 (5 ng/mL)	Sample 1	Sample 5
C	Standard 3 (2.5 ng/mL)	Standard 3 (2.5 ng/mL)	Sample 2	Sample 6
D	Standard 4 (1.25 ng /mL)	Standard 4 (1.25 ng /mL)	Sample 2	Sample 6
E	Standard 5 (0.625 ng /mL)	Standard 5 (0.625 ng /mL)	Sample 3	Sample 7
F	Standard 6 (0.3125 ng/mL)	Standard 6 (0.3125 ng/mL)	Sample 3	Sample 7
G	Standard 7 (0.15625 ng/mL)	Standard 7 (0.15625 ng/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

10. Internal quality control

- The average absorbance of Blank: ≦ 0.15
- The average absorbance of highest concentration of standard (100 ng/mL): ≧ 1.00

11. Calculation of results

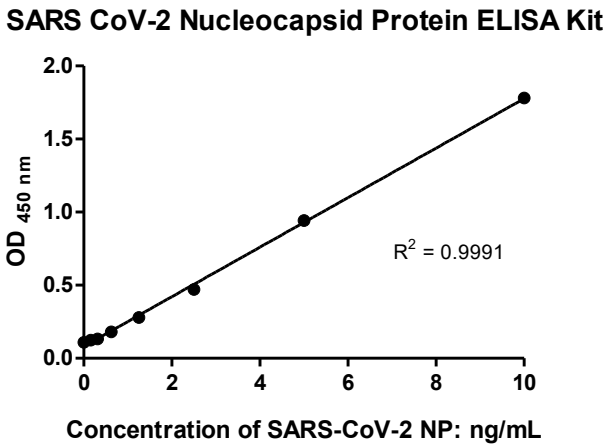
- The linear regression of standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis).
- The SARS CoV-2 NP concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 10 ng/mL, the samples should be diluted with Standard & Sample diluent buffer and re-assay again.
- The actual concentration of sample should be multiplied by the dilution factor.

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Typical data

The following data are for demonstration only

Standard	SARS CoV-2 NP concentration (ng/mL)	OD $_{450\ nm}$
1	10	1.792
2	5	0.979
3	2.5	0.485
4	1.25	0.283
5	0.625	0.191
6	0.3125	0.133
7	0.15625	0.119
Blank	Blank	0.117



12. Assay limitations

- Sample should be centrifuged to remove debris.

13. Performance characteristics

Sensitivity

- The limit of detection (LoD) of this SARS CoV-2 Nucleocapsid Protein ELISA kit is 0.171 ng/mL.
- The limit of quantification (LoQ) of this SARS CoV-2 Nucleocapsid Protein ELISA kit is 0.262 ng/mL.

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