SARS-CoV-2 (2019-nCoV) Nucleoprotein (NP) ELISA Kit Cat. # T3237

For the quantitation of human SARS-CoV-2 (2019-nCoV) NP Antigen concentrations in cell culture supernates, serum, and plasma.

INTRODUCTION

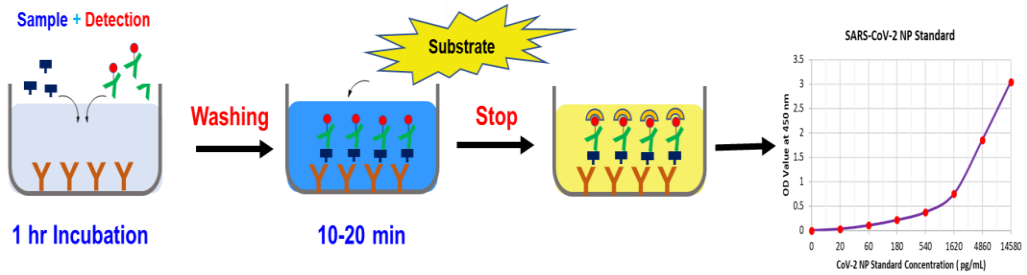
Coronaviruses are enveloped viruses with a positive-sense RNA genome and a nucleocapsid of helical symmetry. Coronavirus nucleoproteins (NP) localize to the cytoplasm and the nucleolus, a subnuclear structure, in both virus-infected primary cells and cells transfected with plasmids that express N protein. Coronavirus N protein is required for coronavirus RNA synthesis, and has RNA chaperone activity that may be involved in template switch. Nucleocapsid protein is a most abundant protein of coronavirus. During virion assembly, N protein binds to viral RNA and leads to formation of the helical nucleocapsid. Nucleocapsid protein is a highly immunogenic phosphoprotein. It is involved in viral genome replication and in modulating cell signaling pathways. Because of the conservation of N protein sequence and its strong immunogenicity, the N protein of coronavirus is chosen as a diagnostic marker.

The Fast SARS-CoV-2 (2019-nCoV) NP ELISA is a solid phase ELISA designed to measure SARS-CoV-2 NP levels in cell culture supernatants, serum, and plasma. The main feature is that it uses our novel proprietary approaches to combine samples and detection into a 1-step to replace the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in about 1.5 hours, not 5-6 hours (Fig. 1). The detection arrangement is from 20 to 14580pg/ml. The levels of SARS-Cov-2 NP in samples are in parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for NP in samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique. A monoclonal antibody specific for SARS-CoV-2 NP was pre-coated onto a microplate. Standards and samples are pipetted into the wells, then incubated with HRP-conjugated detection antibody specific for NP. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of NP bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1 Novel ELISA Procedures



NAME	PART #	DESCRIPTION	STORAGE CONDITIONS
SARS-CoV-2 NP Microplate	TBS3230A	96 well strip microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for SARS-CoV-2 NP.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
SARS-CoV-2 NP Standard	TBS3230B	120 µL of Recombinant SARS-CoV-2 NP (145.8ng/mL)	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3230C	2.2 ml of HRP-SARS-CoV2 NP antibody.	May be stored for up to 3 months at 2-8 °C. Store the unopened kit at 2-8 °C. Do not use after the expiration date. The kit contains sufficient materials to run an ELISA on one 96 well plate.
Assay Diluent	TBS3230D	12 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

PRECAUTIONS

1. Please follow biosafety level 2 guidelines when handling virus samples before sample lysis buffer treatment.
2. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risk.
3. Handle and discard all biological materials as potentially hazardous things by following local laws and regulations.
4. Personal protective items such as lab coats, gloves, surgical masks and goggles are necessary for safety reasons.

REAGENT PREPARATION

Bring all reagents to the room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

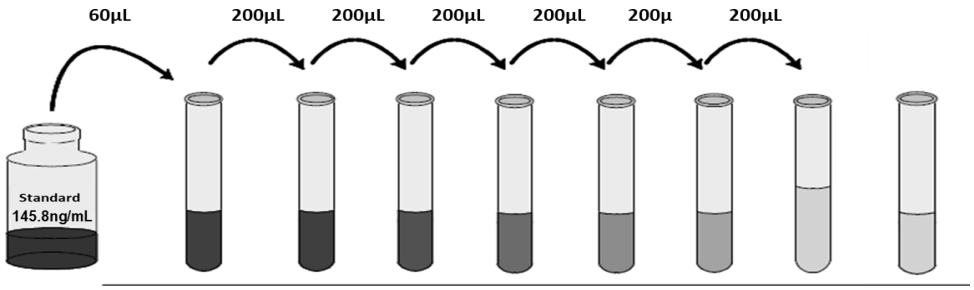
SARS-CoV-2 NP Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 540 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below (Fig. 2).**

2. Add 60 µL of the SARS-CoV-2 NP Standard stock solution (145.8ng/mL) by dilution of 10 times to tube #1 and mix completely.

3. Take 200 µL of the SARS-CoV-2 NP standard from tube #1 to tube #2 and mix completely. Repeat 3 x serial dilutions for tubes #3 through #7. The standard concentration in tube 1 through 7 will be 4860, 1,620, 540, 180, 60, 20 and 6.67pg/mL. Tube# 8 is Standard 0.

Fig. 2 Diagram for SARS-CoV2_NP Standard Preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Diluent (µL)	540	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol (µL)	60	200	200	200	200	200	200	
Std Conc (pg/mL)	14580	4860	1620	540	180	60	20	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour.**
3. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl **TMB Substrate** to each well. Incubate **at RT for 10-20 min.** (*Protect from light*). It becomes blue.
5. Add 50 µl of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).

6. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the SARS-CoV-2 NP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

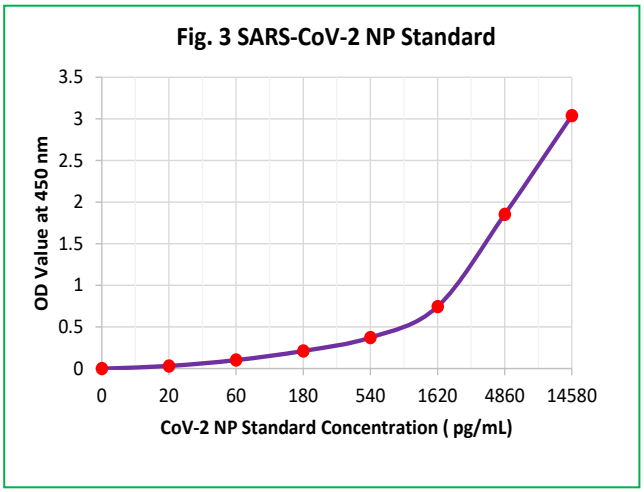
TYPICAL DATA: This standard curve is provided for demonstration only as Fig.3. A standard curve should be generated for each set of samples assayed.

SENSITIVITY: The minimum detectable dose (MOD) of SARS-CoV-2 NP is typically 8pg/ml.

SPECIFICITY : This assay recognizes natural and recombinant SARS-CoV-2 NP.

RELATED PRODUCTS

Human IL-1ß ELISA (T3219)
Human IL-2 ELISA (T3220)
Human IL-4 ELISA (T3221)
Human IL-6 ELISA (T3223)
Human IL-7 ELISA (T3224)
Human IL-8 ELISA (T3225)
Human IL-10 ELISA (T3226)
Human IL-13 ELISA (T3227)
Human IL-17 ELISA (T3228)
Human IL-22 ELISA (T3229)
Human IFN-gamma ELISA (T3230)
Human TGF- ß1 ELISA (T3232)
Human GM-CSF ELISA (T3233)
Human MIP-1α ELISA (T3234)
Human TNFα ELISA(T5235)
Human Insulin ELISA(T3236)
SARS-CoV-2 Spike Antigen ELISA(T3238)
Human IL-18 ELISA(T3239)
Protein Cell Lysis Buffer (catalog# T5001)
Protein Assay Kit (Catalog# T2005)
TMB Substrate System (Catalog#T5021)



All Products are for research use only.