**A Novel Dual Rapid ELISA for sequential detection of Anti-SARS-CoV2 nucleocapsid anti anti-SARS-CoV2 spike**

 **antibodies in the same sample**

 ***Ultrasensitive detection of antibodies to both SARS-CoV2 nucleocapsid and spike protein in 40 mins***

 **FOR RESEARCH USE ONLY- NOT FDA APPROVED**

 Size -96 Assays

 Sample: 50- 100 ul

 Detection: Bioluminescence

 Sensitivity- 10 pg/ml

 Standard curve range 10 pg/ml to 1ug/ml

 Instrument required: Microplate luminometer

 Assay Time: 30 mins

 **ELISA type- Bioluminescence-linked ELISA (BL-ELISA)**

 Catalog # DUAL SNAb-RELISA-01, $650 (Enquire for bulk purchase)

 Advantages:

* Rapid ELISA- Quantitation of both antibodies is complete in about 40min
* Improved sensitivity due to ultrasensitive bioluminescent reporters for detection
* Since both analytes can be measured in the same plate this assay saves time and is cost-effective

Concentration of Anti-SARS-CoV2 nucleocapsid protein antibody

**Description:**

**Application:** This immunoassay kit allows for in vitro quantitative determination of human anti-SARS-CoV2 nucleocapsid antibodies and anti-SARS-CoV2 spike protein antibody concentrations in serum, saliva and other biological fluids. The kit can be stored at 4o C for 6 months

**Sensitivity:** <10 pg/ml

**Additional information:** Direct bioluminescence-linked ELISA, ultrasensitive detection using Gaussia luciferase (to detect

SARS-CoV2 spike protein) and Cypridina luciferase to detect the SARS-CoV2 N protein

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| **Recovery:** Matrices listed below were spiked with certain level of Human anti-SARS-CoV2 (N) IgG and the recovery rates were calculated by comparing the measured value to the expected amount of Human anti-SARS-CoV2 (N) IgG in samples. Enquire for more information. |  |
| **Linearity:**The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human anti-SARS-CoV2 (N) IgG and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected. Enquire for more information. |  |
| Intra-Assay: CV<8%Inter-Assay: CV<12%**CV(%):**  |

**KIT COMPONENTS**

Warning: **Do not use any reagents where damage to the packaging has occurred.**

The kit contains the following reagents:

1. Protein G-coated ELISA **microplate** in a resealable foil pouch, containing 96 polystyrene microtiter wells coated with Protein G in each well. Stable at 2-8°C until the expiration date. Storage: 4 o C for 6 months
2. **10X Detection Probe for detecting antibodies to the SARS-COV2-2 N protein and Spike protein:** One vial (500 ul) . Stable at -20°C until the expiration date. The detection probe for the nucleocapsid antibody is a Luciferase-SARS-CoV2--Nucleocapsid Cypridina Luciferase fusion protein. The detection probe for the spike protein antibody is a Gauusia Luciferase -SARS-CoV2- Spike protein (full length). The detection probe is diluted 10-fold with Buffer 1 just before use. Storage: -80o C for 6 months
3. **Buffer 1:** One bottle, 100 ml. Storage: 4 o C for 6 months
4. **Buffer 2 :** One bottle, 50 mL (used as wash buffer and detection probe dilution buffer). Storage: 4 o C for 6 months. Storage: 4 o C for 6 months
5. **Luciferase assay reagents** (100 assays each of Cypridina luciferase assay reagent (VLAR-2) and the Gaussia luciferase assay reagent (GAR-*Quench and Glo TM*). Storage: -20 o C for 6 months

**MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT**

* Microplate Luminometer
* Single- and multichannel pipettors
* Polypropylene tubes or 96 well dilution plates
* Parafilm or plate sealer, Timer

**WARNINGS AND PRECAUTIONS**

* **This is a “research use only” kit.** This test has not been FDA cleared or approved;

PROTOCOL:

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

2. Add 50 μL diluted samples to each well We recommend a 1: 20 or higher dilution in wash buffer 1

4. Cover with the adhesive strip provided. Incubate for 10 mins at RT

5. Wash once with 300 ul Wash Buffer1. Incubate 1 min

6. Aspirate wash buffer 1 and add 50 ul of diluted Antibody detection probe mix and incubate for 5 min

7. Aspirate antibody detection probe

8. Wash 2X with 300 ul of Wash buffer 2 and IX with Wash buffer 1. Incubate with each wash for 1 min before aspirating

9. After aspirating last wash add 50 ul of Cypridina luciferase assay reagent (VLAR-2 ) and read immediately in a microplate luminometer (integrate for 2 sec/well. Wait 5 min. Add 50 ul Gaussia luciferase assay reagent (GAR-*Quench and Glo TM*) and read ina. Micorpalte luminometer,(integrate for 2 sec/well). **NOTE:** Working Luciferase assay reagent is prepared jus tbefore use by diluting the 100X substrate 100-fold in the respective luciferase assay dilution buffer

**Calculations Of Results**

1. Average the readings for each standard, control and sample, and subtract the background reading

2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean luciferase activity for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the nucleocapsid or Spike S1 Protein concentrations versus the log of the luciferase activity on a linear scale, and the best fit line can be determined by regression analysis.

3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.