SARS-CoV-2 Nucleocapsid Protein Binding Kit (for rabbit antibody)

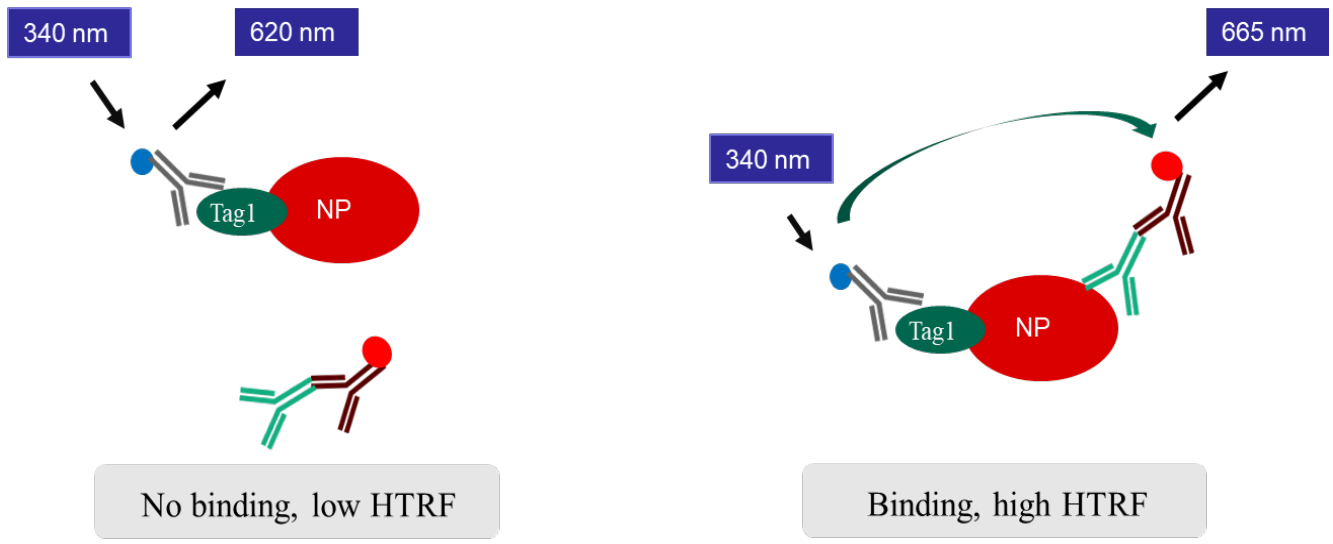
Catalog Number: 728273

Background

SARS-CoV-2 Nucleocapsid protein (NP) is one of the core components of SARS-CoV-2 virus. It forms a complex with viral genomic RNA in a helical symmetrical structure and plays a key role in the process of virus replication and assembly. Since NP is abundantly expressed during infection, it can be used as an important diagnostic marker for COVID-19 and also can be used as a potential drug target or developing vaccines.

Assay Principle

The SARS-CoV-2 Nucleocapsid protein (NP) Binding kit is a TR-FRET based assay, that is designed to detect binding status of NP to the test antibody. Terbium-labeled anti-Tag1 antibody serves as fluorescence donor, that binds to the His-Tagged NP. If a test rabbit antibody binds to NP, fluorescence-labeled anti-rabbit antibody (fluorescence acceptor) will be brought in close proximity with the fluorescence donor. Excitation of Terbium (340 nm) generates fluorescence resonance energy transfer (FRET) to the fluorescence-labeled acceptor, which consequently fluoresces at 665 nm (figure below). Thus, the test antibody binding to NP can be quantitively measured by calculation of the fluorescent ratio of 665 nm/620 nm.



Application

High throughput screening of antibodies that bind to NP.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
728262	2x Assay Buffer	25 mL	-20°C
728271	Recombinant SARS-CoV-2 Nucleocapsid protein (full length)	5 µL	-80°C
447852	Terbium-labeled anti-Tag1 Ab	20 µL	-80°C
727952	fluorescence-labeled anti-rabbit antibody	20 µL	-80°C
	384-well microplate	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. Customer Test anti-NP-rabbit antibody (to be tested antibody)
3. 0.5 M DTT
4. Adjustable micro-pipettor
5. Sterile Tips

Assay protocol

1. Prepare 1X assay buffer containing 1 mM DTT (1X DTT-containing assay buffer)

   For example, mix 996 µl distilled water with 1000 µl of 2X assay Buffer (Catalogue number: XXXXXX) and 4 µl of 0.5 M DTT. Make only enough 1X DTT-containing assay buffer as needed for the assay. Store the remaining 2X assay buffer at -20°C.

2. Prepare SARS-CoV-2 Nucleocapsid protein

   Dilute SARS-CoV-2 Nucleocapsid protein (NP) 1,500-fold with 1X DTT-containing assay buffer. For example: 1 µl of NP + 1,499 µl of 1X DTT-containing assay buffer.

   Add 5 µl of diluted NP protein to each well.

3. Prepare Antibody solution

Prepare mouse antibody with 1X DTT-containing assay buffer to the concentration to be tested.

Add 5 µl of diluted antibody solution to each well except negative control wells.

4. Prepare dye solution

   Dilute Terbium-labeled anti-Tag1 Ab and fluorescence-labeled anti-rabbit antibody 1:200 in 1X DTT-containing assay buffer. For example: 1 µl of Terbium-labeled anti-Tag1 Ab + 1 µl of fluorescence-labeled anti-rabbit antibody + 198 µl of 1X DTT-containing assay buffer.

   Add 10 µl of this dye mixture to each well.

5. Incubate the reaction at room temperature for 1 hour.

6. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples. Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Antibody Test
1X DTT-containing assay buffer	5 µl	-
Diluted NP solution	5 µl	5 µl
Diluted antibody solution	-	5 µl
Tb-Anti-Rabbit Ab + Anti-Tag1 Ab	10 µl	10 µl
Total Volume	20 µl	20 µl
Incubate at room temperature for 1 hour.		

Data Analysis

1. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio1 = \frac{\text{Fluorescent intensity at 620 nm}}{\text{Fluorescent intensity at 340 nm}}$$

2. Calculate the ratio of the fluorescent intensity of each well.

$$Ratio2 = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 340 nm}}$$

3. Calculate sample signal.

$$Sample\ signal = \frac{Ratio2}{\text{Ratio1}}$$

4. Calculate percentage activity

In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\% \text{ Activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

Data Presentation