Description

The RdRp (SARS-CoV-2) TR-FRET Assay Kit is designed to measure the activity of the SARS-CoV-2 RNA-dependent RNA Polymerase (RdRp) for screening and profiling applications. RdRp operates as a complex of NSP12, NSP7, and NSP8 proteins. As a crucial enzyme in the life cycle of coronaviruses, RdRp represents one of the most promising druggable targets for SARS-CoV-2. The RdRp (SARS-CoV-2) TR-FRET Assay Kit comes in a convenient 384-well format, with Digoxigenin-labeled RNA duplex, biotinylated ATP, RdRp assay buffer (2 components plus DTT), and purified RdRp complex. The assay measures the direct incorporation of biotinylated ATP into the double-stranded RNA substrate. The increase in TR-FRET signal is proportional to the amount of ATP incorporated in the RNA. With this homogeneous kit, only two steps are required for RdRp activity detection. First, a test compound is incubated with the enzyme in the reaction mixture. Next, Dye-labeled Acceptor and Eu-labeled Antibody Donor are added, followed by reading of the TR-FRET signal.

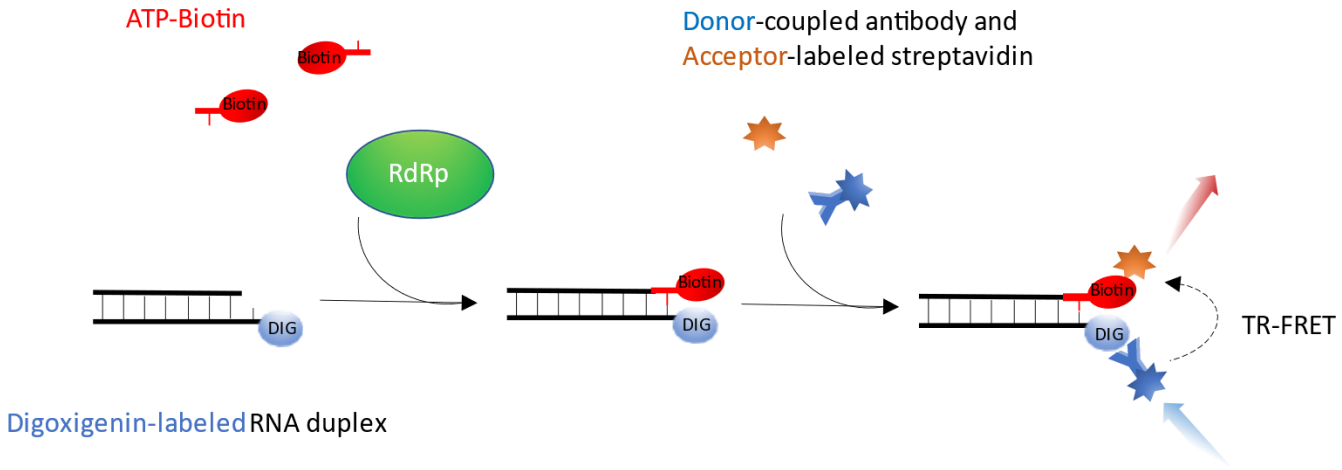

Figure 1: RdRp (SARS-CoV-2) TR-FRET Assay Kit schematic.

Application(s)

Study enzyme kinetics and use in high throughput screening (HTS) applications.

Supplied Materials

This kit contains an RNA substrate. It is critical to wear gloves and use RNAse-free conditions.

Catalog #	Name	Amount	Storage
101466	NSP12/NSP7/NSP8 (SARS-CoV-2) Complex*	2 x 100 µg	-80°C
	Digoxigenin-labeled RNA Duplex	2 x 4 µl	-80°C
	Biotinylated ATP	2 x 4 µl	-80°C
	RNAse inhibitor	100 µl	-80°C
	RdRp assay buffer component 1 (Incomplete Buffer)	2 x 5 ml	-20°C
	RdRp assay buffer component 2 (Incomplete Buffer)	2 x 20 µl	-20°C
	DTT (0.5 M)	2 x 200 µl	-20°C
	Eu-labeled anti-Digoxigenin Antibody	2 x 4 µl	-80°C
	Dye-labeled acceptor	2 x 10 µl	-20°C
	RdRp TR-FRET Detection buffer**	5 ml	-20°C
	384-well Plate with Plate sealer	1	Room Temp.

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*
***Contains a toxic compound. Use appropriate precautions*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/thaw cycles.***

Safety

This product is for research purposes only and not for human or therapeutic use. **The TR-FRET detection reagent contains a toxic compound. Use appropriate precautions.** This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating antibodies or protein inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

Use RNase-free conditions for all steps!

Step 1:

1. Prepare complete RdRp buffer: First, add 10 µl of 0.5M DTT to 5 ml of RdRp Assay Buffer Component 1 (Incomplete buffer 1). Mix well. Then add 20 µl of RdRp Assay Buffer Component 2. Mix well again.

   *Note: Many of the reagents in this assay kit **cannot be reused** after thawing or preparation. BPS Bioscience has provided these reagents split into two vials so that the researcher can use half the plate at one time and half the plate on a different day. **Do not reuse** thawed reagents or thawed DTT.*
   All amounts indicated in the protocol use one vial of each reagent to assay half the plate.

2. Dilute the RNAse inhibitor 8-fold with complete RdRp buffer. Prepare only enough inhibitor as is required for the assay; store the remaining inhibitor at -20°C.

3. Thaw one vial of **RdRp** on ice. Briefly spin the tube containing the enzyme to recover its full contents.

   *Note: RdRp is very sensitive to freeze/thaw cycles**. Do not reuse** the thawed vial or the diluted enzyme.*

4. Dilute **RdRp** in complete RdRp buffer at 125 ng/µl (you will need 4 µl of diluted enzyme per well: 500 ng/4 µl). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme.

5. Add 4 µl of diluted **RdRp** to each well designated “Positive Control” and “Test Inhibitor”. For the "Blank", add 4 µl of Complete RdRp buffer.

Component	Blank	Positive Control	Test Sample
Complete RdRp buffer	4 µl	-	-
Diluted RdRp complex (125 ng/µl)	-	4 µl	4 µl
Diluted RNAse Inhibitor	2 µl	2 µl	2 µl
Test Inhibitor	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
RdRp Reaction Mixture	2 µl	2 µl	2 µl
Total	10 µl	10 µl	10 µl

6. Add 2 µl of diluted RNAse Inhibitor to each well.

7. Prepare the test inhibitor (2 µl/well): For a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations in Complete RdRp Buffer. The final volume of the reaction is 10 µl.

   Without DMSO
   a. If the Test Inhibitor is water-soluble, prepare serial dilutions in the Complete RdRp Buffer, 5-fold more concentrated than the desired final concentrations. For the positive and negative controls, use Complete RdRp Buffer (Diluent Solution).

   Or

   With DMSO
   a. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 50-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in Complete RdRp Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

b. Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in Complete RdRp Buffer to keep the concentration of DMSO constant.

c. For positive and negative controls, prepare 5% DMSO in Complete RdRp Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).
The final concentration of DMSO in the assay should be ≤ 1% DMSO.

8. Add 2 µl of inhibitor serial dilutions to each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 2 µl of the Diluent solution (without inhibitor).
Preincubate for 30 minutes at room temperature with slow shaking on a rotator platform.

9. During RdRp preincubation with the inhibitor(s), prepare the RdRP Reaction Mixture as follows:

a. Dilute one vial of Digoxigenin-labeled RNA duplex 50-fold in Complete RdRp Buffer

b. Dilute one vial of Biotinylated ATP 50-fold in Complete RdRp Buffer

10. Prepare the reaction mixture using diluted reagents: N=200 wells (half plate) x (1 µl of diluted Digoxigenin-labeled RNA Duplex + 200 µl of diluted Biotinylated ATP).

11. Initiate the reaction by adding 2 µl of diluted RdRp Reaction Mixture prepared as described above. Seal the wells with a plate sealer. Incubate for one hour at 37°C.

Step 2:

1. Thaw the TR-FRET Detection Buffer on ice.

2. Dilute one vial of Eu-labeled antibody (1:600) and one vial of Dye-labeled acceptor (1:200) using the TR-FRET Detection Buffer in one mix:

3. Add 3.3 µl of Eu-labeled antibody and 10 µl of Dye-labeled acceptor to 1987 µl of TR-FRET Detection buffer.

Note: A mix of 2 ml is sufficient for half of the 384-well plate.

4. Add 10 µl of Antibody/Acceptor mixture per well.

5. Place on rotating platform for 10-30 minutes at room temperature.

6. Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET.
Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	317±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

Example Results

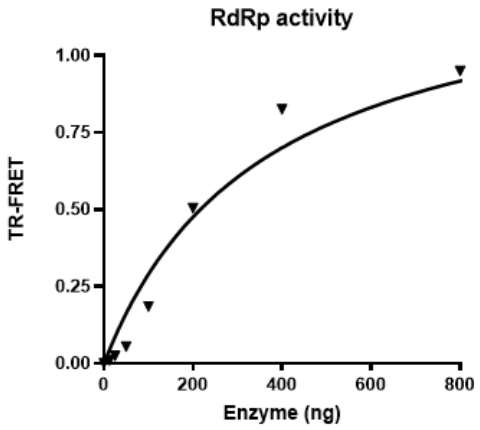

Figure 2: RdRp Activity.
Titration of RdRp enzyme activity using increasing amounts of NSP8_NSP7/NSP12 (SARS-CoV-2) Complex Recombinant (BPS Bioscience #101466)

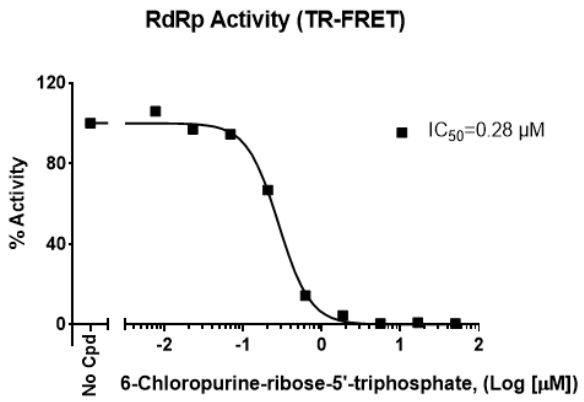

Figure 3: RdRp Activity (TR-Fret).
RdRp inhibition by increasing concentrations of 6-Chloropurine-ribose triphosphate (right), measured using RdRp (SARS-CoV-2) TR-FRET Assay Kit (BPS Bioscience #78553).

Results are representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General considerations
"Blank" Control: The "Blank" control is important to determine the background TR-FRET in the assay.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
RdRp/NSP7/NSP8 (SARS-CoV-2) Complex	101466	10 µg
RNA Polymerase, FLAG-tag (SARS-CoV-2)	100729	100 µg
NSP7, His-tag (SARS-CoV-2)	100829-1	100 µg
NSP7, His-tag (SARS-CoV-2)	100829-2	1 mg
NSP8, His-tag (SARS-CoV-2)	100830-1	100 mg
NSP8, His-tag (SARS-CoV-2)	100830-2	1 mg
NSP10/NSP16 Complex (SARS-CoV-2)	100747-1	100 µg