Description

The RdRp (SARS-CoV-2) TR-FRET Assay Kit is a TR-FRET (Time-resolved fluorescence energy transfer) designed to measure the activity of the SARS-CoV-2 RNA-dependent RNA Polymerase (RdRp) for screening and profiling applications. The RdRp (SARS-CoV-2) TR-FRET Assay Kit comes in a convenient 96-well format, with Digoxigenin-labeled RNA duplex, biotinylated ATP, RdRp assay buffer (2 components plus DTT), and purified RdRp complex.

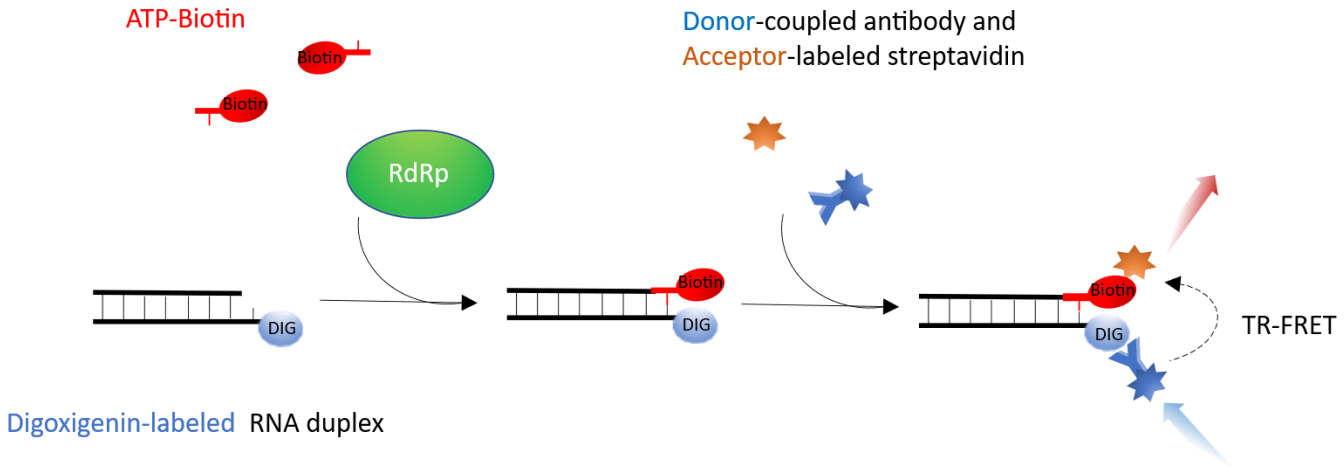

Figure 1: RdRp (SARS-CoV-2) TR-FRET Assay Kit schematic.

The assay measures the direct incorporation of biotinylated ATP into the double-stranded RNA substrate. 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. The increase in TR-FRET signal is proportional to the amount of ATP incorporated in the RNA.

Background:

SARS-CoV-2 RNA-dependent RNA Polymerase (RdRp) operates as a complex of NSP12, NSP7, and NSP8 proteins. It is crucial enzyme in the life cycle of the coronovirus-2 (SARS-CoV-2) as it is involved in transcription and replication of the virus. The global pandemic of 2019 has brought attention to the potential of targeting RdRp. It represents one of the most promising druggable targets for SARS-CoV-2, and its inhibition by remdesivir was the first FDA-approved agent for the treatment of this disease.

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	NSP8_NSP7/NSP12 (SARS-CoV-2) Complex*	100 µg	-80°C
82858	Digoxigenin-labeled RNA Duplex	4 µl	-80°C
82859	Biotinylated ATP	4 µl	-80°C
82860	RNAse Inhibitor	50 µl	-80°C
82861	RdRp Assay Buffer Component 1 (Incomplete Buffer)	5 ml	-20°C
82862	RdRp Assay Buffer Component 2 (Incomplete Buffer)	20 µl	-20°C
82735	0.5 M DTT	200 µl	-20°C
	Eu-labeled Anti-Digoxigenin Antibody	4 µl	-80°C
	Dye-labeled Acceptor	10 µl	-20°C
82863	RdRp TR-FRET Detection Buffer**	5 ml	-20°C
79685	Black 96-well plate	1	Room Temp.
82610	Plate sealing film	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
- Rotating or rocker platform
- Nuclease-free water
- Incubator set to 37°C

Storage Conditions

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

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.

Contraindications

- This kit is compatible with up to 1% final DMSO concentration.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor".
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using 6-Chloropurine-ribose triphosphate as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC_{50} value shown in the validation data below.
- 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.

Work under RNase-free conditions for all steps! We recommend cleaning the lab bench and pipettors with an RNase decontamination solution (e.g. RNase*Zap*™ wipes, Thermo Fisher #AM9786) before you start.

Step 1:

1. Prepare **Complete RdRp Buffer**:
    a. Add 10 µl of **0.5M DTT** to 5 ml of **RdRp Assay Buffer Component 1 (Incomplete Buffer)**.
    b. Mix well.
    c. Add 20 µl of **RdRp Assay Buffer Component 2 (Incomplete Buffer)**.
    d. Mix well again.
2. Dilute the **RNAse Inhibitor** 8-fold with Complete RdRp Buffer (4 µl/ well).
3. Thaw **RdRp complex** on ice. Briefly spin the tube containing the enzyme to recover its full content.
4. Dilute **RdRp complex** with Complete RdRp Buffer to 125 ng/µl (8 µl/well). Keep the diluted enzyme on ice until use.
5. Add 8 µl of diluted **RdRp complex** to each well designated "Positive Control" and "Test Inhibitor".
6. For the "Blank" wells, add 8 µl of Complete RdRp Buffer.
7. Add 4 µl of diluted RNAse Inhibitor to each well.
8. Prepare the **Test Inhibitor** (4 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 20 µl.

8.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 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

8.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in Complete RdRp Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

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.

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).

Note: The final concentration of DMSO in the assay should be ≤ 1% DMSO.

9. Add 4 µl of inhibitor serial dilutions to each well designated “Test Inhibitor”.

10. For the “Positive Control" and "Blank" add 4 µl of the Diluent solution (without inhibitor).

11. Preincubate for 30 minutes at RT (Room Temperature) with slow agitation on a rotator platform.

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

    a. Dilute **Digoxigenin-labeled RNA Duplex** 50-fold with Complete RdRp Buffer.

    b. Dilute **Biotinylated ATP** 250-fold with Complete RdRp Buffer.

    c. For 100 wells, prepare 200 µl of diluted Digoxigenin-labeled RNA Duplex + 200 µl of diluted Biotinylated ATP.

13. Initiate the reaction by adding 4 µl of RdRp Reaction Mixture prepared as described above.

Component	**Blank**	**Positive Control**	**Test Sample**
Complete RdRp Buffer	8 µl	-	-
Diluted RdRp complex (125 ng/µl)	-	8 µl	8 µl
Diluted RNAse Inhibitor	4 µl	4 µl	4 µl
Test Inhibitor	-	-	4 µl
Diluent Solution	4 µl	4 µl	-
RdRp Reaction Mixture	4 µl	4 µl	4 µl
Total	**20 µl**	**20 µl**	**20 µl**

14. Seal the wells with a plate sealer. Incubate for one hour at 37°C.

Step 2:

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

2. Prepare an **Antibody/Acceptor Mix** by diluting together Eu-labeled antibody 600-fold and Dye-labeled acceptor 200-fold with TR-FRET Detection Buffer as follows:

    a. 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 a whole plate.

3. Add 20 µl of Antibody/Acceptor Mix per well.

4. Place on rotating platform for 10-30 minutes at RT.

5. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument).

6. The "Blank" value should be subtracted from all other values.

Instrument Settings

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

Reading Mode	Time Resolved
Excitation Wavelength	317 (20 nm bandwidth)
Emission Wavelength	620 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317 (20 nm bandwidth)
Emission Wavelength	665 (10 nm bandwidth)
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).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{blank}}{FRET_P - FRET_{blank}} \times 100\%$$

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

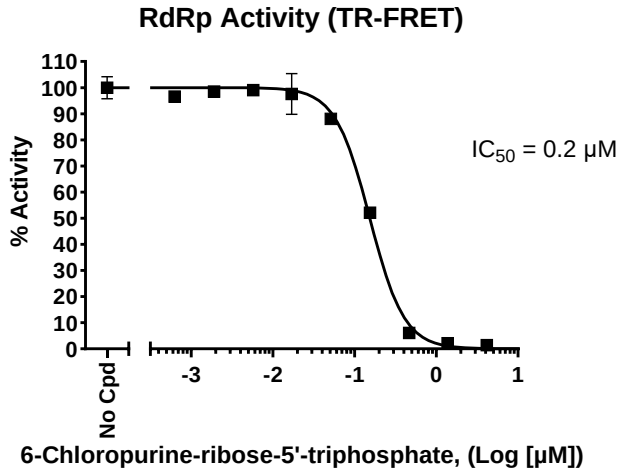

Figure 2: RdRp activity is inhibited by 6-Chloropurine-ribose triphosphate.
RdRp inhibition was measured in the presence of by increasing concentrations of 6-Chloropurine-ribose triphosphate.

Results are representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Wang Z., *et al.*, 2022 *Front Immunol.* 13: 1015355.

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

Version 061125