Description

The ADAR1:RNA TR-FRET Assay Kit is designed to measure the binding of ADAR1 (adenosine deaminase, RNA-specific 1) to RNA for screening and profiling applications using TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer). It utilizes Terbium-labeled donor and dye-labeled acceptor to complete the TR-FRET pairing. The ADAR1:RNA TR-FRET Assay Kit comes in a convenient 384-well format, with enough purified ADAR1, Tb-Labeled Donor and Dye-Labeled Acceptor, RNase inhibitor, ADAR1 substrate and assay buffer for 384 reactions. The assay also includes a Competitor RNA as control.

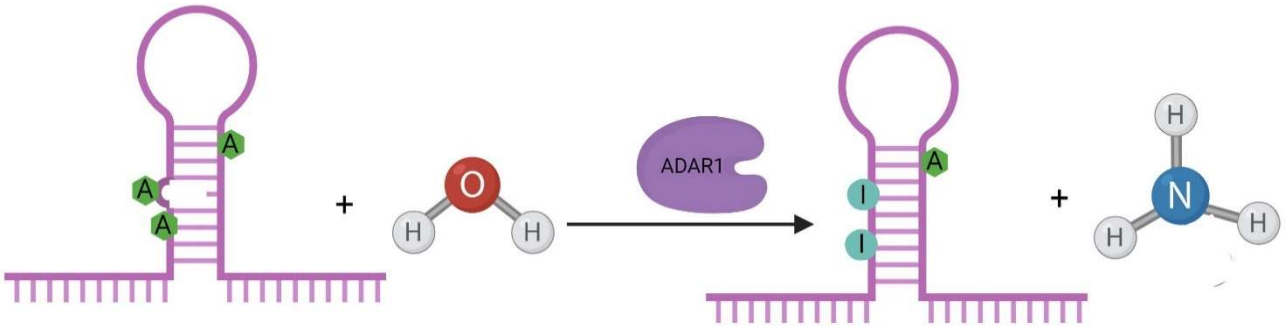

Figure 1: ADAR1 mechanism of action on RNA molecules.

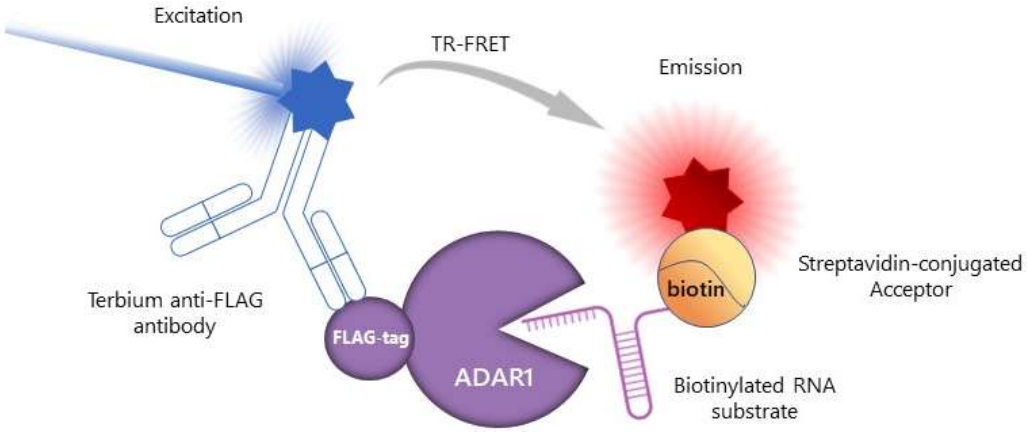

Figure 2: Assay principle.

The Terbium-labeled anti-FLAG antibody donor binds to FLAG-tagged ADAR1, while the dye-labeled streptavidin acceptor binds to the biotinylated RNA substrate. When ADAR1 forms a complex with its RNA substrate TR-FRET occurs and can be measured using a TR-FRET fluorescence plate reader. TR-FRET signal is proportional to RNA binding.

Background

ADAR1 (adenosine deaminase, RNA-specific 1) performs adenosine to inosine base editing in RNA, particularly targeting adenosines located within a specific stem-loop motif structure. It is proposed that ADARs evolved to provide additional diversity to the transcriptome and while the majority of ADAR editing events occur in non-coding RNAs, some, including the canonical GluA2 editing site, alter the amino acid sequence of coding proteins. ADAR1 plays a role in innate immunity by mitigating interferon signaling. Dysfunction of ADAR1 results in autoimmune disorders, and impacts cancer cell growth and proliferation as well as tumor response to immunotherapy. Since ADAR recognizes double-stranded RNA, it also functions to suppress or modify RNA viruses. Thus, it is implicated in viral evolution and in the emergence of viral variants such as SARS-CoV-2 variants.

Application(s)
Study RNA-binding kinetics and use in high throughput screening (HTS) applications.

Supplied Materials

This kit contains an RNA substrate. It is critical to wear gloves and work under RNase-free conditions.

Catalog #	Name	Amount	Storage
100472	ADAR1, FLAG-Tag*	3 µg	-80°C
	ADAR1 Substrate, Biotin-Labeled (10 µM)	20 µl	-80°C
	RNase Inhibitor	100 µl	-80°C
	Competitor RNA (10 µM)	10 µl	-80°C
	3x ADAR Binding Buffer	3 ml	-20°C
	Anti-FLAG Tb-Labeled Donor	2 x 10 µl	-20°C
	Dye-Labeled Acceptor	10 µl	-20°C
79969	White 384-well microplate	1	Room Temp

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
- Incubator set to 30°C
- Nuclease-free water
- Adjustable micropipettor and sterile filter tips
- Rotating or rocker platform

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. 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.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control”, ‘Inhibitor 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 RNA Competitor 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 IC50 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 1x ADAR1 Binding Buffer by diluting **3x ADAR1 Binding Buffer** 3-fold with nuclease-free water.

2. Dilute **RNAse Inhibitor** 8-fold with 1x ADAR1 Binding Buffer (2 µl/well).

   Note: Prepare only enough inhibitor as is required for the assay and store the remaining inhibitor at -80°C.

3. Thaw **ADAR1** on ice. Briefly spin the tube containing the enzyme to recover its full content.

4. Dilute **ADAR1** to 1.75 ng/µl with 1x ADAR1 Binding buffer (4 µl/well).

5. Add 4 µl of diluted **ADAR1** to each well designated “Positive Control”, “Inhibitor Control” and “Test Inhibitor”.

6. Add 4 µl of 1x ADAR1 Binding buffer to the "Blank" wells.

7. Add 2 µl of diluted **RNase Inhibitor** to each well.

8. Prepare the test inhibitor (2 µl/well): For a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations in 1x ADAR1 Binding Buffer. The final volume of the reaction is 10 µl.

   8.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in 1x ADAR1 Binding Buffer, 5-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x ADAR1 Binding 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 100 % DMSO, then dilute the inhibitor 20-fold in 1x ADAR1 Binding 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 1x ADAR1 Binding Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% DMSO in 1x ADAR1 Binding Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO in the assay should not exceed 1%.

9. Add 2 µl of the inhibitor serial dilutions to each well designated "Test Inhibitor".

10. Add 2 µl of the Diluent solution to the "Positive Control" and "Blank" wells.

11. Prepare the Inhibitor Control by diluting the **Competitor RNA (10 µM)** to a value that is 50x the IC_{50} in 1x ADAR1 Binding Buffer (2 µl/well). Using 1x ADAR1 Binding Buffer, prepare solutions at 0.5x and 5x the IC_{50} value (2 µl/well).

12. Add 2 µl of diluted **Competitor RNA** to the "Inhibitor Control" wells.

13. Incubate at 30°C for 30 minutes.

    Note: We strongly recommend preincubation of the enzyme with the inhibitor before adding the substrate.

14. Dilute **ADAR1 Substrate, Biotin-Labeled (10 µM)** 100-fold with 1x ADAR1 Binding Buffer (2 µl/well). This makes a 100 nM **ADAR1 Substrate Solution**.

    Note: Dilute only enough substrate required for the assay. Store remaining ADAR1 Substrate, Biotin-Labeled (10 µM) at -80°C in single-use aliquots (5 µl minimum volume per aliquot).

15. Bring diluted **ADAR1 Substrate Solution** to 30°C in an incubator set to 30°C.

16. Initiate the reaction by adding 2 µl of prewarmed diluted **ADAR1 Substrate Solution** to each well.

17. Incubate at 30°C for 15 minutes.

Component	Blank	Positive Control	Inhibitor Control	Test Inhibitor
1x ADAR1 Binding Buffer	4 µl	-	-	-
Diluted ADAR1 (1.75 ng/µl)	-	4 µl	4 µl	4 µl
Diluted RNase Inhibitor	2 µl	2 µl	2 µl	2 µl
Test Inhibitor	-	-	-	2 µl
Diluted Competitor RNA	-	-	2 µl	-
Diluent Solution	2 µl	2 µl	-	-
Incubate at 30°C for 30 minutes				
Diluted ADAR1 Substrate Solution (100 nM)	2 µl	2 µl	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

Step 2:

1. Dilute **Anti-FLAG Tb-Labeled Donor** 300-fold and **Dye-Labeled Acceptor** 600-fold, together, with 1x ADAR1 Binding Buffer (you will need 10 µl of Donor/Acceptor Mix/well)

   Note: Make only enough as needed for the assay; store the remaining stock solution in aliquots at -20°C.

2. Add 10 µl of Donor/Acceptor Mixture to each well.

3. Incubate at room temperature for 30-60 minutes with slow agitation.

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

5. 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	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Calculate the FRET value by using the following formula:

$$FRET = \frac{S_{665} - \left(\frac{Tb_{665}}{Tb_{620}} \times S_{620}\right)}{S_{620}} \times 1000$$

S_{665} = Sample value measured at 665 nm, S_{620} = Sample value measured at 620 nm, Tb_{665} = Tb only or Blank value measured at 665 nm, Tb_{520} = Tb only or Blank value measured at 520 nm.

The FRET value calculated for the negative control should be subtracted from all other measurements and can be set as 0%. The FRET value from the "Positive Control" can be set as 100% activity.

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

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

Example Results

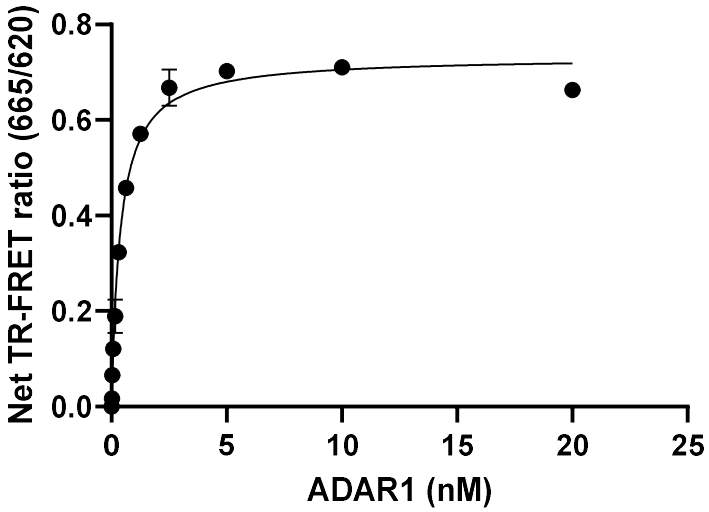

Figure 3: ADAR1:RNA binding.
Increasing amounts of ADAR1 enzyme were incubated with 20 nM ADAR1 Substrate, Biotin-Labeled. The binding of ADAR1 to RNA TR-FRET signal was measured using a Tecan microplate reader.

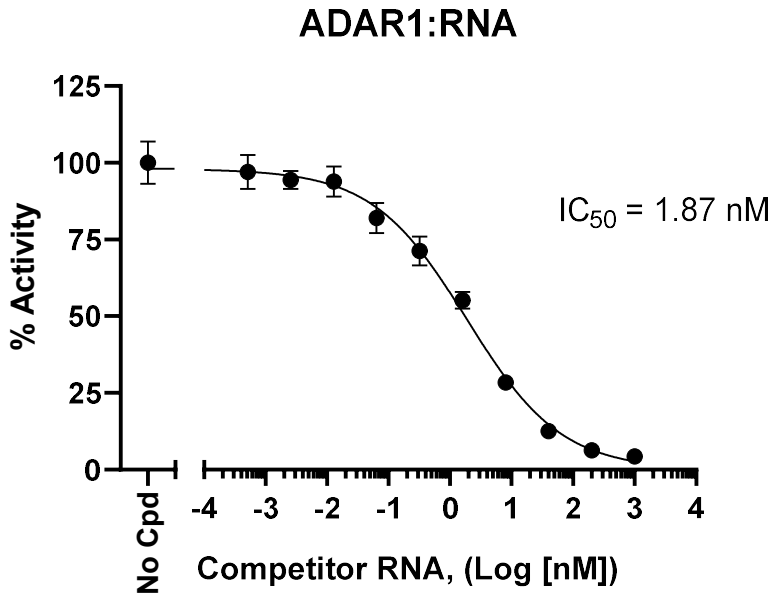

Figure 4: ADAR:RNA binding is inhibited by Competitor RNA.
ADAR1 binding to RNA was measured in the presence of increasing concentrations of Competitor RNA (Inhibitor Control). TR-FRET signal was measured using a Tecan microplate reader.

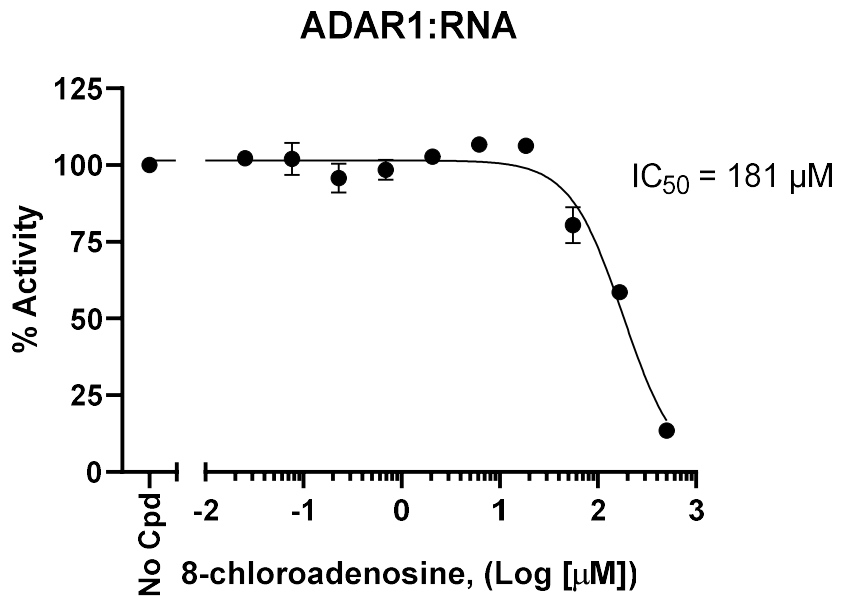

Figure 5: ADAR:RNA binding is inhibited by 8-chloroadenosine.
ADAR1 binding to RNA was measured in the presence of increasing concentrations of 8-chloroadenosine (Tocris #4436). TR-FRET signal was measured using a Tecan microplate reader.

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

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

References
Keegan L. P., *et al.,* 2023 *Acc Chem Res* 56 (22): 3165-3174.
Mendoza H. G., *et al.,* 2023 *Biochemistry* 62 (8): 1367-1387.

Related Products

Products	*Catalog #*	*Size*
ADAR2 (ADARB1), FLAG-Tag Recombinant	101164	10 µg
Adenosine Deaminase (ADA), His-Tag Recombinant	70016	100 µg
DPP4 Assay Kit	80204	96 reactions
DPP4, His-tag Recombinant	80040	10 µg

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