Description

ADAR1 Activity TWO-Luciferase Reporter HEK293 Cell Line is a HEK293 cell line designed to monitor cell viability in parallel with ADAR1 (adenosine deaminase acting on RNA) enzyme activity. These cells were engineered to express ADAR1 (NM_001111.5) and an ADAR1 reporter construct. This construct is comprised of an ADAR1 hairpin target with a stop codon (UAG) susceptible to ADAR1-mediated editing to tryptophan (UUG), located upstream of a firefly luciferase reporter. In addition, they constitutively express Renilla Luciferase under the control of a CMV promoter, which can be used to determine cell viability (Figure 1).

This cell line has been validated by treatment with ADAR1 siRNA and the ADAR1 inhibitor Fludarabine.

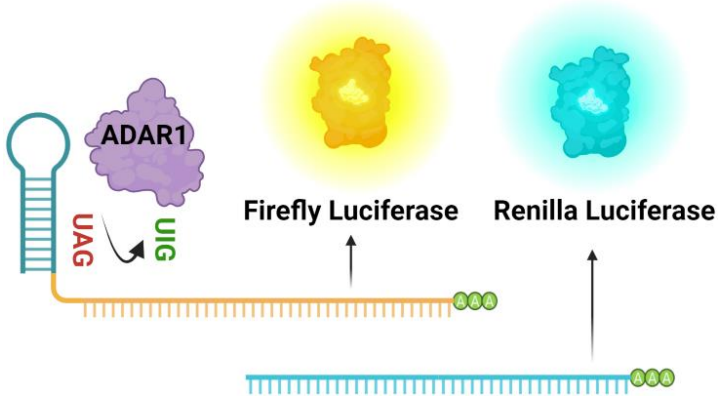

Figure 1: Illustration of the mechanism of action of ADAR1 Activity TWO-Luciferase Reporter HEK293 Cell Line.
The ADAR1 reporter construct is comprised of an ADAR1 hairpin target with a stop codon (UAG) upstream of the sequence encoding firefly luciferase. In the absence of ADAR1, firefly luciferase is not transcribed, and the cells show no firefly luciferase activity. However, these cells were engineered to express ADAR1, which converts adenine into inosine, now encoding the amino acid tryptophan (UUG) and enabling transcription and expression of firefly luciferase. ADAR1 activity, therefore, directly correlates with firefly luciferase activity. Renilla luciferase is constitutively expressed from a separate construct and therefore Renilla luciferase activity correlates with the number of cells, but not with ADAR1 activity.

Background

ADAR (Adenosine Deaminase Acting on RNA) enzymes perform adenosine to inosine base editing in RNA, particularly targeting adenosines located within a specific double-stranded stem-loop motif (Figure 1). In the context of healthy, uninfected cells, ADAR1 performs A-to-I editing on endogenous double-stranded RNA to prevent it from activating the downstream dsRNA sensors RIG-I (retinoic acid-inducible gene I) and MDA5 (melanoma differentiation-associated protein 5), which in-turn activate a pro-inflammatory response. Loss-of-function mutations in ADAR1 result in aberrant activation of the dsRNA sensors and are involved in autoimmune disorders. ADAR1 dysfunction also impacts cancer cell growth, proliferation, and response to immunotherapy. ADAR1 expression is increased in many tumor types and ADAR1 knock-out has been demonstrated to improve the response to certain immunotherapies such PD-1 (programmed death protein 1)/PD-L1 (programmed death ligand 1) blockade and to circumvent tumor immunotherapy resistance mechanisms, making ADAR1 an attractive target for therapeutic development.

Application

- Monitor ADAR1 activity.
- Study the effect of compounds on ADAR1 activity.
- Simultaneous measurement of compound effect on ADAR1 activity and impact on cell viability.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1Y	BPS Bioscience #82535

Materials Required for Cellular Assays

Name	Ordering Information
ADAR1 Responsive Luciferase Reporter HEK293 Cell Line	BPS Bioscience #82238
ADAR1 Targeting siRNA	Horizon #M-008630-01-0005
Lipofectamine RNAi Max	Thermo Fisher #13778030
Assay Medium 1A	BPS Bioscience #79805
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Fludarabine	BPS Bioscience #82534
96-well tissue culture white, clear-bottom assay plate	Corning #3610
TWO-Step Luciferase (Firefly and Renilla) Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage.
Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin

Growth Medium 1Y (BPS Bioscience # #82535):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 0.5 µg/ml of Puromycin, 100 µg/ml Hygromycin B and 400 µg/ml G418.

Assay Medium:
Thaw Medium 1 (BPS Bioscience #60187)
Assay Medium 1A (BPS Bioscience #79805)

Cell Culture Protocol
Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1Y.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Y and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1Y.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1Y and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~1 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Functional Validation

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A should include "ADAR1 Activity TWO-Luciferase Reporter Cells" and "Background Control" conditions.
- Assay B should include "siRNA Treated", "Non-Transfected Control" and "Background Control" conditions.
- Assay C should include "Compound Treated", "Untreated Control" and "Background Control" conditions.

A. ADAR1 Activity TWO-Luciferase Reporter Cell Line cell titration curve.

1. Seed ADAR1 Activity TWO-Luciferase Reporter HEK293 cells at varying cell densities in 100 µl of Thaw Medium 1 into a white clear-bottom 96-well cell culture plate. For a cell titration curve, we recommend performing a serial dilution at 2: 1 cells to media. Leave a few empty wells as "Background Control" wells.

2. Incubate the cells at 37°C with 5% CO_2 for 24 hours.

3. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.

4. Incubate with gentle agitation at RT for ~15 to 30 minutes.

5. Measure Firefly luminescence using a luminometer.

6. On the same plate, add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.

7. Measure Renilla luminescence using a luminometer.

8. Data Analysis: Subtract the respective background luminescence from both the Firefly and Renilla luminescence reading of all conditions. Next, for each well, divide the Firefly luminescence signal by the Renilla luminescence signal.

$$Normalized\ Luminescence = \left(\frac{Firefly\ luminescence\ of\ cells - Firefly\ luminescence\ background}{Renilla\ luminescence\ of\ cells - Renilla\ luminescence\ background}\right)$$

Note: The normalized luminescence signal may vary between experiments based upon the luminometer and luminometer settings used to read the Firefly and Renilla luminescence signals. Depending upon the instrument used it may be necessary to read the plate at different gain settings for the Firefly vs Renilla luciferase reads.

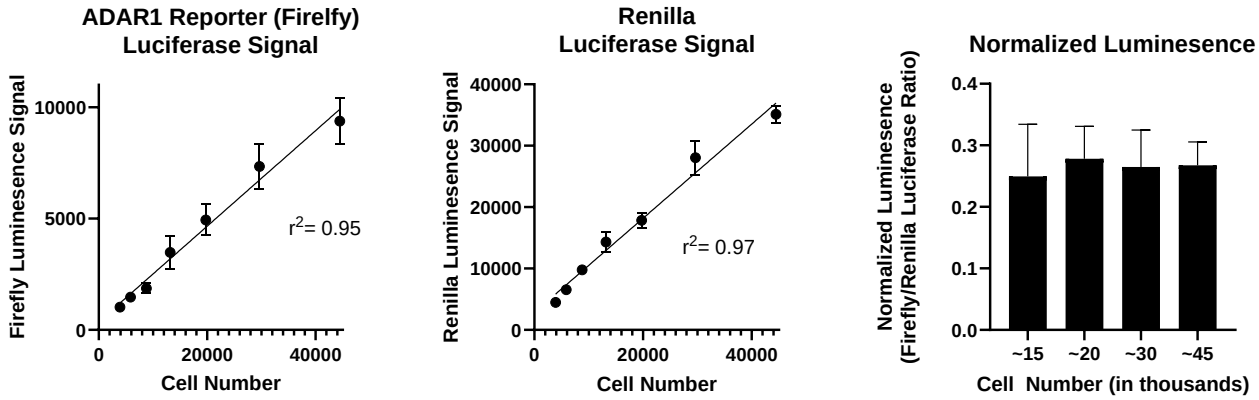

Figure 2. Luciferase activity in a cell titration curve for ADAR1 Activity TWO-Luciferase Reporter Cell Line.
Cells were plated at various densities in a 96-well plate overnight. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results of both the background-subtracted luminescence signal (left and middle panels) and normalized luminescence signal (right panel) are shown.

B. Inhibition of ADAR1 activity with ADAR1 siRNA in ADAR1 Activity TWO-Luciferase Reporter Cell Line.

1. Seed ADAR1 Activity TWO-Luciferase Reporter HEK293 cells at a density of 20,000 cells/well in 90 µl of Thaw Medium 1 into a white clear-bottom 96-well cell culture plate. Leave a few empty wells as "Background Control" wells.

2. Prepare several concentrations of siRNA for transfection, according to the manufacturer instructions (10 µl/well).

3. Prepare a Lipofectamine RNAiMax/ Assay Medium 1A (no siRNA) mix (10 µl/well of "Non-Transfected Control").

4. Add 10 µl of the prepared siRNA solutions to each of the "siRNA Treated" wells.

5. Add 10 µl of Lipofectamine RNAiMax/ Assay Medium 1A mix to the "No-Transfected Control" wells.

6. Incubate the cells at 37°C with 5% CO_2 for 24 hours.
7. Remove the cell culture medium from the cells and replace it with 100 µl of Thaw Medium 1.

8. Incubate the cells at 37°C with 5% CO_2 for 48 hours.
9. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.
10. Incubate with gentle agitation at RT for ~15 to 30 minutes.
11. Measure Firefly luminescence using a luminometer.
12. Add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.
13. Measure Renilla luminescence using a luminometer.
14. Data Analysis: Subtract the background luminescence from both the Firefly and Renilla luminescence reading of all conditions. Next, calculate the Normalized Luminescence for each well by dividing the background-subtracted Firefly luminescence signal by the background-subtracted Renilla luminescence signal. The percent normalized luminescence is the normalized luminescence of the siRNA-treated cells divided by the normalized luminescence of untreated control cells ("Non-Transfected Controls"), multiplied by 100.
    No Transfected Control is set at 100%.

$$Normalized\ Luminescence = \left(\frac{Firefly\ luminescence\ of\ cells - Firefly\ luminescence\ background}{Renilla\ luminescence\ of\ cells - Renilla\ luminescence\ background}\right)$$

$$Percent\ Normalized\ Luminescence = \left(\frac{luminescence\ of\ siRNA\ treated\ cells}{luminescence\ of\ Non-Transfected\ Control\ cells}\right) x100$$

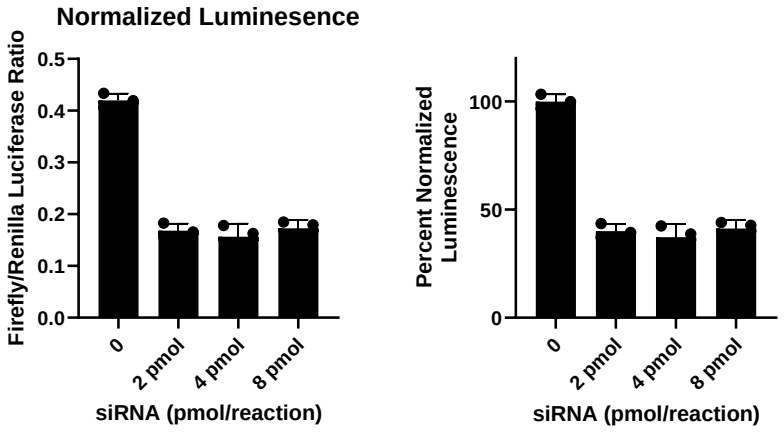

Figure 3. Inhibition of ADAR1 activity by an ADAR1-targeting siRNA in ADAR1 Activity TWO-Luciferase Reporter HEK293 Cell Line.
Cells were transfected with an ADAR1-targeting siRNA for 24 hours. The media was replaced with new media and cells were then incubated for 48 hours. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results are shown as both normalized luminescence (left panel) and percentage normalized luminescence (right panel) compared to cells without siRNA treatment (set to 100%).

C. Inhibition of ADAR1 activity with small molecule inhibitors in ADAR1 Activity TWO-Luciferase Reporter Cell Line.

1. Seed ADAR1 Activity TWO-Luciferase Reporter HEK293 cells at a density of 40,000 cells/well in 100 µl of Thaw Medium 1 into a white clear-bottom 96-well cell culture plate. Leave a few empty wells as "Background Control" wells.
2. Incubate the cells at 37°C with 5% CO_2 for 24 hours.
3. Prepare a 3-fold increment serial dilution of Test Compound in Thaw Medium 1 (100 µl/well).
4. Remove the cell culture medium from the cells.
5. Add 100 µl of diluted Test Compound to the "Compound Treated" wells.
6. Add 100 µl of Thaw Medium 1 to "Untreated Control" wells.
7. Add 100 µl of Thaw Medium 1 to "Background Luminescence" wells.
8. Incubate at 37°C with 5% CO_2 for 24 hours.
9. Remove the cell culture medium from the cells and replace it with new 100 µl of Thaw Medium 1.
10. Incubate the cells at 37°C with 5% CO_2 for 48 hours.
11. Add 100 µl per well of TWO-Step Firefly Luciferase Assay Working Solution.
12. Incubate with gentle agitation at RT for ~15 to 30 minutes.
13. Measure Firefly luminescence using a luminometer.
14. Add 100 µl per well of TWO-Step Renilla Luciferase Assay Working Solution.
15. Measure Renilla luminescence using a luminometer.
16. Data Analysis: Subtract the background luminescence from both the Firefly and Renilla luminescence reading of all conditions. Next, calculate the Normalized Luminescence for each well by dividing the background-subtracted Firefly luminescence signal by the background-subtracted Renilla luminescence signal. The percent normalized luminescence is the normalized luminescence of the siRNA-treated cells divided by the normalized luminescence of untreated control cells ("Untreated Control"), multiplied by 100.
    Untreated Control is set at 100%.

$$Normalized\ Luminescence = \left(\frac{Firefly\ luminescence\ of\ cells - Firefly\ luminescence\ background}{Renilla\ luminescence\ of\ cells - Renilla\ luminescence\ background}\right)$$

$$Percent\ Normalized\ Luminescence = \left(\frac{luminescence\ of\ Compound\ treated\ cells}{luminescence\ of\ Untreated\ control\ cells}\right) x100$$

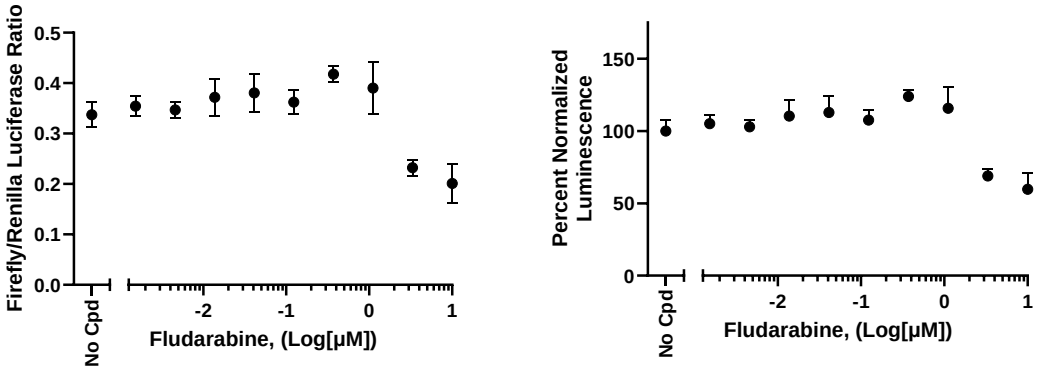

Figure 3. Inhibition of ADAR1 activity by the small molecule inhibitor Fludarabine in ADAR1 Activity TWO-Luciferase Reporter HEK293 Cell Line.
The day following cell plating, cells were treated with the ADAR1 inhibitor Fludarabine for 24 hours. Both Firefly and Renilla luciferase activity were measured using TWO-Step Luciferase (Firefly & Renilla) Assay System. Results are shown as both normalized luminescence (left panel) and percentage normalized luminescence (right panel) compared to cells without inhibitor treatment (set to 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

References
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Buchumenski I., *et al.*, 2019 *Nature* 565(7737):43-48.
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Yuan J., *et al.,* 2023 *J Exp Clin Cancer Res* 42: 149.
Zhang T., *et al.,* 2022 *Nature* 606: 594–602.

Related Products

Products	*Catalog #*	*Size*
ADAR1 Responsive Luciferase Reporter HEK293 Cell Line	82238	2 vials
ADAR1 Activity Luciferase Reporter HEK293 Cell Line	82239	2 vials
ADAR1, FLAG-Tag Recombinant	100472	50 µg/100 µg
ADAR2 (ADARB1), FLAG-Tag	101164	10 µg
ADAR1: RNA TR-FRET Assay Kit	82252	384 reactions

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