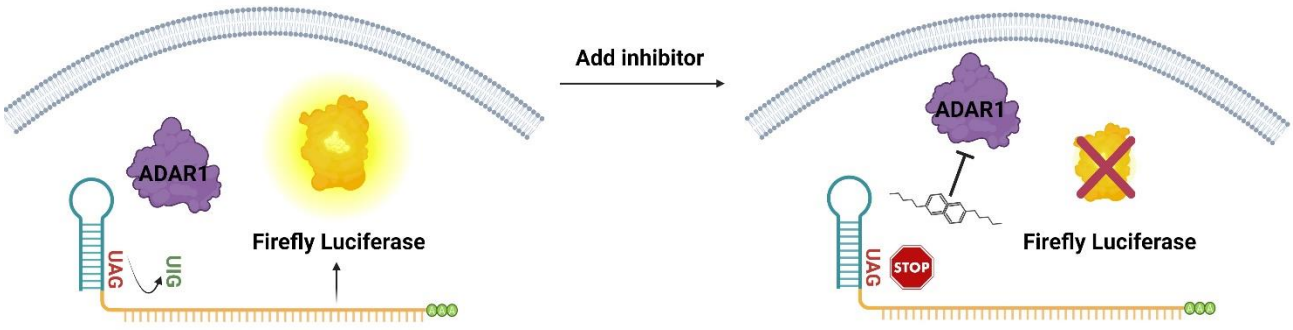
Description

ADAR1 Activity Luciferase Reporter HEK293 Cell Line is a HEK293 cell line designed to monitor the activity of ADAR1 (adenosine deaminase acting on RNA) enzyme. These cells were engineered to over-express human ADAR1 (NM_001111.5) and an ADAR1 reporter construct 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 (*Figure 1*).

This cell line has been validated by comparing reporter activation to ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (#82238) and in response to treatment with ADAR1 siRNA.



Created with BioRender.com

Figure 1: Illustration of the mechanism of action of ADAR1 Activity 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 luciferase. In the absence of ADAR1, luciferase is not transcribed, and the cells show no luciferase activity. In the presence of ADAR1 activity, adenine is converted into inosine, encoding now the amino acid tryptophan (UUG) and enabling transcription and expression of luciferase. ADAR1 activity, therefore, directly correlates with luciferase 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 shown 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.

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 1U	BPS Bioscience #78548

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™ RNAiMAX Transfection Reagent	Thermo Fisher #13778030
Assay Medium 1A	BPS Bioscience #79805
Thaw Medium 1	BPS Bioscience #60187
96-well tissue culture white, clear-bottom assay plate	Corning #3610
ONE-Step™ Luciferase 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 1U (BPS Bioscience # 78548):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 0.5 µg/ml of Puromycin and 100 µg/ml Hygromycin B.

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 1U.

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 1U 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 1U.

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 1U and count the cells.

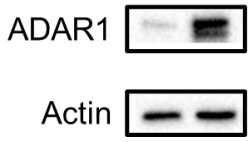
3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~1 x 10^6 cells/ml.

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.

Validation Data



Lane 1: ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (Parental Control)
Lane 2: ADAR1 Activity Luciferase Reporter HEK293 Cell Line (ADAR1 OE)

Figure 2. Western Blot analysis of ADAR1 protein expression in ADAR1 Activity Luciferase Reporter HEK293 Cell Line.
ADAR1 Activity Luciferase Reporter HEK293 cell lysates or parental ADAR1 Responsive Luciferase Reporter HEK293 cell lysates were analyzed by Western Blot using either Recombinant Anti-ADAR1 antibody [EPR7033] (Abcam #ab126745) or β-Actin (13E5) Rabbit mAB (Cell Signaling Technology #4970) primary antibodies, followed by Secondary Anti-HRP-labeled Antibody 2 (BPS Biosciences #52131H).

Functional Validation

- The following assay was designed for a 96-well or 384-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 and B should include "ADAR1 Activity Reporter Cells", "Background Control" and "Parental Reporter Only" conditions.
- Assay C should include "siRNA Treated", "Non-Transfected Control" and "Background Control" conditions.
- We recommend not using the edge wells of the plate and use interior wells only.

A. Activity comparison of ADAR1 Activity Reporter Cell Line and ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (Parental "Reporter Only" Cell Line) in a 96-well format.

1. Seed ADAR1 Activity Luciferase Reporter HEK293 cells at a density of 40,000 cells/well in 100 µl of Thaw Medium 1 into half of the test wells of a white clear-bottom 96-well cell culture plate. Leave a few empty wells as "Background Control" wells.

2. Seed parental ADAR1 Responsive Luciferase Reporter HEK293 ("Parental Reporter Only") cells at a density of 40,000 cells/well in 100 µl of Thaw Medium into the other half of the test wells of a white clear-bottom 96-well cell culture plate.

3. Add 100 µl of Assay Medium to "Background Control" (cell free wells).

4. Incubate the cells at 37°C with 5% CO_2 overnight.

5. Add 100 µl of ONE-Step™ Luciferase reagent per well.

6. Incubate with gentle agitation at Room Temperature (RT) for ~15 minutes.

7. Measure luminescence using a luminometer.

8. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells, excluding edge wells. The Z' and % CV statistics can be calculated as outlined below.

$$Z' = 1 - \left(\frac{(3\ x\ standard\ deviation\ positive\ sample) + (3\ x\ standard\ deviation\ negative\ control)}{absolute\ value\ (mean\ of\ positive\ sample - mean\ of\ negative\ control)}\right)$$

$$\%\ CV = \left(\frac{Standard\ Deviation\ Sample}{Mean\ Sample}\right) x100$$

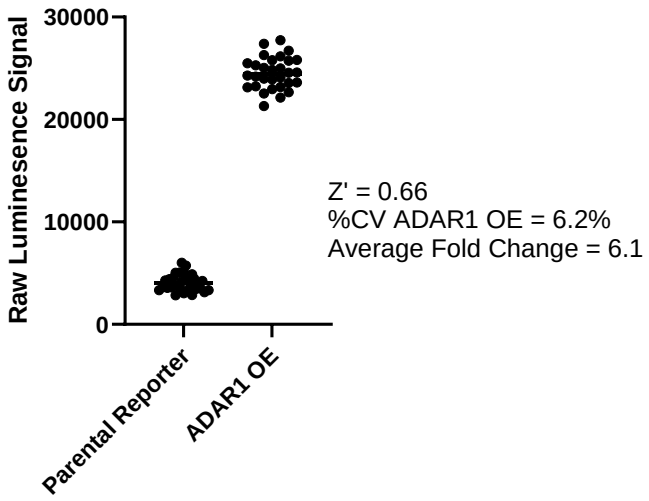


Figure 3. Luciferase *activity in ADAR1 Activity Luciferase Reporter HEK293 Cell Line (ADAR1 OE) versus the parental ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (Parental Reporter).* Cells were plated in a 96-well plate overnight. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as raw luminescence signal.

B. Activity comparison of ADAR1 Activity Reporter Cell Line and ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (Parental "Reporter Only" Cell Line) in a 384-well format.

1. Seed ADAR1 Activity Luciferase Reporter HEK293 cells at a density of 5,000 cells/well in 40 µl of Thaw Medium 1 into half of the test wells of a white clear-bottom 384-well cell culture plate. Leave a few empty wells as "Background Control" wells.

2. Seed parental ADAR1 Responsive Luciferase Reporter HEK293 cells ("Parental Reporter Only") at a density of 5,000 cells/well in 40 µl of Thaw Medium into the other half of the test wells of a white 384-well cell culture plate.

3. Add 40 µl of Assay Medium to "Background Control" (cell free wells).

4. Incubate the cells at 37°C with 5% CO_2 overnight.

5. Add 40 µl of ONE-Step™ Luciferase reagent per well.

6. Incubate with gentle agitation at RT for ~15 minutes.

7. Measure luminescence using a luminometer.

8. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells, excluding edge wells. The Z' and % CV statistics can be calculated as outlined below.

$$Z' = 1 - \left(\frac{(3\ x\ standard\ deviation\ positive\ sample) + (3\ x\ standard\ deviation\ negative\ control)}{absolute\ value\ (mean\ of\ positive\ sample - mean\ of\ negative\ control)}\right)$$

$$\%\ CV = \left(\frac{Standard\ Deviation\ Sample}{Mean\ Sample}\right) x100$$

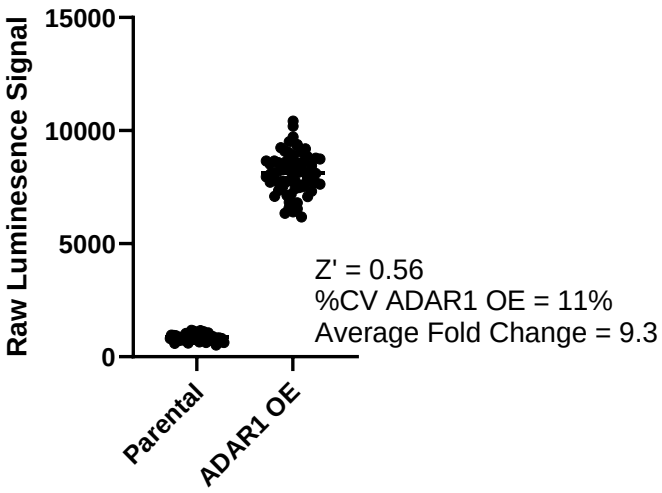


Figure 4. Luciferase *Activity in ADAR1 Activity Luciferase Reporter HEK293 Cell Line (ADAR1 OE) versus parental ADAR1 Responsive Luciferase Reporter HEK293 Cell Line (Parental).*
Cells were plated in a 384-well plate overnight. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as raw luminescence signal.

C. Inhibition of ADAR1 activity by ADAR1 siRNA in ADAR1 Activity Reporter HEK293 Cell Line.

1. Seed ADAR1 Activity 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. Seed additional wells for cytotoxicity assessment, if desired. 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) (10 µl/"Non-Transfected Control" well).

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 "Non-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 ONE-Step™ Luciferase Assay reagent.

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

11. Measure luminescence using a luminometer.

12. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of siRNA-treated cells divided by the background-subtracted luminescence of untreated control cells ("Non-Transfected Control"), multiplied by 100.
    Non-Transfected Control is set at 100%.

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

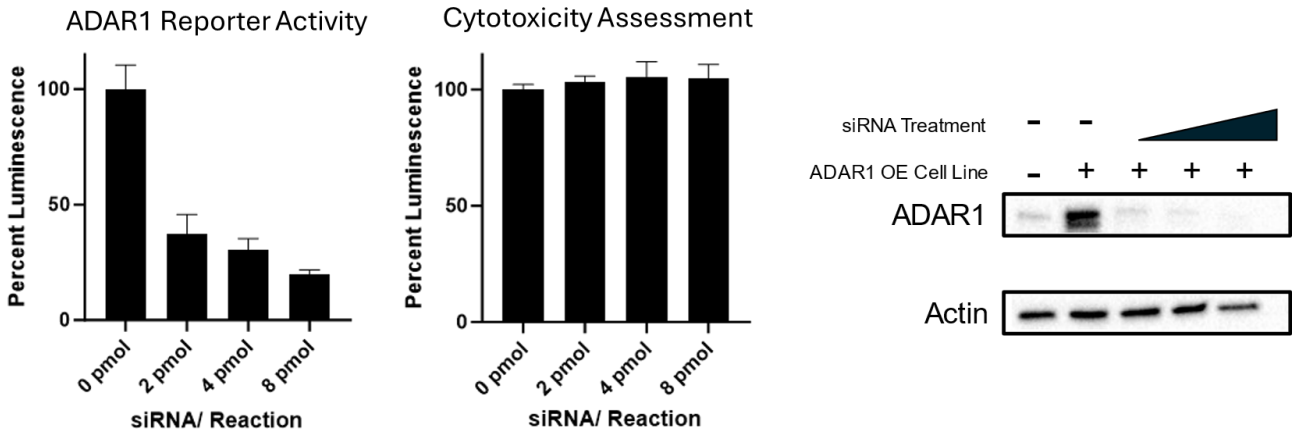


Figure 5. Inhibition of ADAR1 activity by an ADAR1-targeting siRNA in ADAR1 Activity Luciferase Reporter HEK293 Cell Line.
Left Panel: Cells were transfected with an ADAR1-targeting siRNA for 24 hours, followed by a fresh media exchange. Cells were then incubated for a further 48 hours. Luciferase activity was then measured using One-Step™ Luciferase Assay System. Results are shown as percentage luminescence compared to cells without siRNA treatment (set to 100%). Middle panel: Cytotoxicity was assessed with CellTiter Glo® Luminescence Cell Viability Assay (Promega #G7571) after siRNA treatment. Right panel: Western Blot analysis was performed to confirm siRNA-mediated ADAR1 knockdown in ADAR1 Activity Luciferase Reporter HEK293 Cell Line (ADAR1 OE Cell Line). "siRNA Treated" and "Non-Transfected Control" (-/+ condition) ADAR1 Activity Luciferase Reporter HEK293 cell lysates were tested. Parental ADAR1 Responsive Luciferase Reporter cell lysate was included as negative control (-/- condition). Cell lysates were analyzed by Western Blot using either Recombinant Anti-ADAR1 antibody [EPR7033] (Abcam #ab126745) or β-Actin (13E5) Rabbit mAB (Cell Signaling Technology #4970) primary antibodies, followed by Secondary Anti-HRP-labeled Antibody 2 (BPS Biosciences #52131H).

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

License Disclosure
Visit bpsbioscience.com/license for the label license and other key information about this product.

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

References

Bhate A., *et al.*, 2019 *Mol Cell.* 73(5):866-868.
Buchumenski I., *et al.*, 2019 *Nature.* 565(7737):43-48.
Gallo, A., *et al.*, 2017 *Human Genetics* 136(9): 1265–1278.
Ishizuka JJ, Manguso RT, Cheruiyot CK, Bi K, Panda A, Iracheta-Vellve A, Miller BC, Du PP, Yates KB, Dubrot J, Xu L. and Öhman M., 2018 *Genes (Basel).* 10(1):12.
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, FLAG-Tag	100472	50 µg/100 µg
ADAR2 (ADARB1), FLAG-Tag	101164	10 µg
ADAR1:RNA TR-FRET Assay Kit	82252	384 reactions

Version 050124