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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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Description

ADAR1 Responsive Luciferase Reporter HEK293 Cell Line is a HEK293 cell line designed to respond to ADAR1 enzymatic activity. These cells were engineered to express 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 after transfection with ADAR1 and ADAR2. This cell line has been used for the development of the constitutive ADAR1 expressing ADAR1 Activity Luciferase Reporter HEK293 Cell Line (#82239).

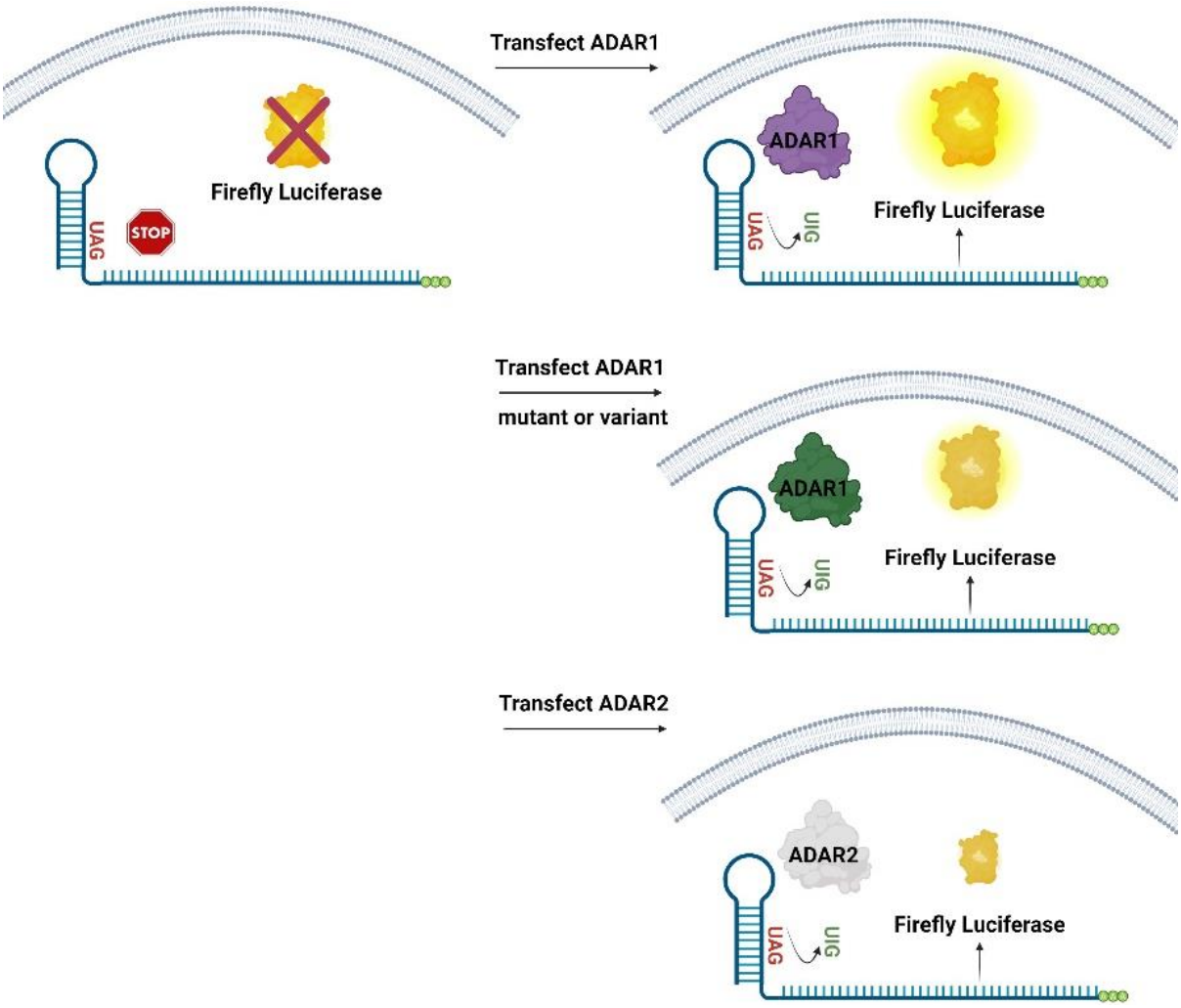

Figure 1: Illustration of the mechanism of action of ADAR1 Responsive Luciferase Reporter HEK293 Cell Line.
The ADAR1 reporter construct is comprised of an ADAR1 hairpin target with a stop codon (UAG) upstream of the sequence encoding luciferase. In the presence of ADAR1 activity, as in the case of transfection with ADAR1, adenine is converted into inosine, encoding now the amino acid tryptophan (UUG) and enabling transcription and expression of luciferase. In the absence of transfected ADAR1, luciferase is not transcribed, and the cells show background luciferase activity. Luciferase activity directly correlates with ADAR1 activity.

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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.

HEK293 cells express low levels of endogenous ADAR1, therefore this cell line is ideal for studies of genetically engineering variants of ADAR1. For example, one can compare the activity of ADAR1 variants introduced into the cells by transfection or transduction. The use of luciferase as reporter allows for an easy assay read-out, making this cell line an attractive cellular system for ADAR1 studies.

Application

- Monitor expressed ADAR1 activity.
- Compare the activity of ADAR1 modifications and mutations in a cellular model, for example in studies on the relationship between structure and function of ADAR1 derivatives for RNA editing therapy.

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 1N	BPS Bioscience #79801

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Materials Required for Cellular Assays

Name	Ordering Information
ADAR1 Activity Luciferase Reporter HEK293 Cell Line	BPS Bioscience #82239
Plasmids encoding ADAR1 isoforms and ADAR2	
Lipofectamine™ 2000 Transfection Reagent	Thermo Fisher #11668027
Assay Media: 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°C 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 1N (BPS Bioscience #79801):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, and 0.5 µg/ml of Puromycin.

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.

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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 1N.

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 1N 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 1N.

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 1N 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.

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Validation Data

- 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.
- The assay should include "ADAR Overexpression" (cells where ADAR1 or ADAR2 was introduced via transfection or other method), "Negative Control" (No addition of ADAR1 or ADAR2, mock transfection condition) and "Background Luminescence" conditions.

A. Comparison of ADAR1 Responsive Luciferase Reporter HEK293 Cell Line activity in response to ADAR1 and ADAR2 overexpression

1. Seed ADAR1 Responsive Luciferase Reporter HEK293 cells at a density of 30,000 cells/well in 90 µl of Thaw Medium 1 into 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. Remove the cell culture medium from the cells and replace with 100 µl of fresh Thaw Medium 1.

4. Prepare several concentrations of the plasmid DNA of interest using Lipofectamine™ 2000 in Assay Medium 1A, according to manufacturer instructions (10 µl/well).

5. Prepare a solution containing including only Lipofectamine™ 2000 and Assay Medium 1A as control (10 µl/"Negative Control" well).

6. Add 10 µl of the prepared plasmid DNA to the "ADAR Overexpression" wells.

7. Add 10 µl of Lipofectamine/Assay Medium 1A mix to the "Negative Control" wells.

8. Add 100 µl of Assay Medium 1A to the "Background Luminescence" wells.

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

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

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

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The fold induction is the background-subtracted luminescence of cells overexpressing ADAR divided by the background-subtracted luminescence of control cells ("Negative Control").
    The Negative Control is set at 1.

$$Fold\ Induction\ = \left(\frac{luminescence\ of\ ADAR\ Overexpressing\ cells - background}{luminescence\ of\ Negative\ Control\ cells - background}\right)$$

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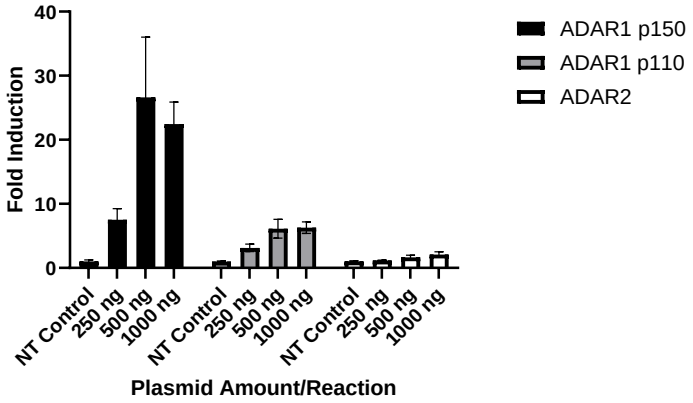

Figure 2. ADAR1 reporter response in ADAR1 Responsive Luciferase Reporter HEK293 Cell Line transfected with different ADAR1 isoforms or ADAR2.
Cells were transfected with plasmids encoding different ADAR1 isoforms (ADAR1 p150 and ADAR1 p110) or ADAR2 for 24 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as fold induction of ADAR1 luciferase reporter activity (compared to cells without ADAR overexpression).

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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Zhang T., *et al.*, 2022 *Nature* 606, 594–602.

Related Products

Products	*Catalog #*	*Size*
ADAR1 Activity Luciferase Reporter HEK293 Cell Line	82239	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

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