FOXO Reporter Kit (PI3K/AKT Pathway)

#60643
500 reactions

Description

The FOXO Reporter Kit (PI3K/AKT Pathway) is designed for monitoring the activity of the PI3K (phosphoinositide 3-kinase)/AKT (protein kinase B) signaling pathway and the transcriptional activity of FOXO (forkhead box O) proteins in a cellular model. The kit contains a transfection-ready FOXO3 Expression Vector, a FOXO Luciferase Reporter Vector, which is a PI3K/AKT pathway-responsive reporter, and a Renilla Luciferase Vector. The FOXO Luciferase Reporter Vector contains the firefly luciferase reporter under the control of multimers of the FOXO responsive element located upstream of a minimal promoter. The FOXO reporter vector comes premixed with a constitutively expressing sea pansy (Renilla) luciferase vector, which serves as an internal control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively expressing Renilla luciferase vector as negative control. The non-inducible luciferase vector also contains the firefly luciferase gene under the control of a minimal promoter, but without the additional FOXO responsive elements. The negative control is critical for determining pathway-specific effects and background luciferase activity.

Background

The (phosphoinositide 3-kinase)/AKT (protein kinase B) signaling pathway is essential for cell growth and survival. Disruption of this pathway or its regulation has been linked to a variety of cancers and coronary diseases. Mammalian FOXO (forkhead box O) proteins (FOXO1, FOXO3, FOXO4), a subgroup of Forkhead transcription factors, is among the best characterized targets of the PI3K/AKT signaling pathway. These transcription factors function as a trigger for apoptosis by up-regulating genes necessary for cell death. Insulin or growth factors induce activation of PI3K, which in turn activates AKT. AKT directly phosphorylates FOXOs, resulting in the export of FOXOs from the nucleus to the cytoplasm, thereby inhibiting FOXO-dependent transcription.

Applications

- Monitor PI3K/AKT signaling pathway activity and FOXO transcriptional activity.
- Screen for compounds that affect PI3K/AKT signaling pathway activity.
- Study effects of RNAi or gene overexpression on the activity of the PI3K/AKT pathway.

Components

Catalog #	Name	Amount	Storage
Component A	Reporter Mix (FOXO Luciferase Reporter Vector + constitutively expressing Renilla Luciferase Vector) (60 ng DNA/µl)	500 µl	-20°C
Component B	Negative Control Reporter Mix (Non-Inducible Luciferase Vector + constitutively expressing Renilla Luciferase Vector) (60 ng DNA/µl)	500 µl	-20°C
Component C	FOXO3 Expression Vector (100 ng DNA/µl)	250 µl	-20°C
Component D	Negative Control Expression Vector (Empty Expression Vector, without FOXO3) (100 ng DNA/µl)	250 µl	-20°C

Note: These vectors are ready for transient cell transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

Materials Used in Cellular Assay

Name	Ordering Information
Mammalian cell line of interest	
Appropriate cell culture media	
Lipofectamine™ 2000 Transfection Reagent	Thermo Fisher #11668027
Opti-MEM™ I Reduced Serum Medium	Invitrogen #31985-062
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
TWO-Step™ Luciferase (Firefly & Renilla) Assay System	BPS Bioscience #60683
Luminometer	

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.

General Transfection and Assay Protocols

- The following procedure is designed to transfect the reporter into HEK293 cells using Lipofectamine™ 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine™ 2000, follow the manufacturer's transfection protocol.
- Transfection conditions should be optimized according to the cell type and study requirements. All amounts and volumes in the following setup are given on a per well basis.
- We recommend setting up at least triplicate assays for each condition and preparing transfection cocktails for multiple wells to minimize pipetting errors.

Day 1:

1. Seed cells at a density of 30,000 cells per well in 100 µl of appropriate cell growth medium.

   Note: Cells should be 90% confluent at the time of transfection.

Day 2:

1. Based on the experimental design of interest, prepare the following DNA transfection mixes, and mix gently:

   a. 1 µl of Component A in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and 1 µl of Component B in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well as transfection control.

b. 1 µl of Component A + experimental vector expressing gene of interest, for example Component C in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and as transfection controls:

   i. 1 µl of Component A + Component D in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,

   ii. 1 µl of Component B + experimental vector expressing gene of interest, for example Component C, in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,

   iii. 1 µl of Component B + Component D in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.

c. 1 µl of Component A + specific siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well, and as transfection controls:

   i. 1 µl of Component A + Negative Control siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,

   ii. 1 µl of Component B + specific siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well,

   iii. 1 µl of Component B + Negative Control siRNA in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.

2. Mix Lipofectamine™ 2000 gently before use, then dilute 0.35 µl of Lipofectamine™ 2000 in 15 µl of Opti-MEM™ I Reduced Serum Medium (no antibiotics) per well.

   Note: Prepare this cocktail in a volume sufficient for the whole experiment.

3. Incubate for 5 minutes at Room Temperature (RT).

4. After the 5 minute incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000 (you should now have 30 µl of solution per experimental well).

5. Mix gently and incubate for 25 minutes at RT.

6. Add 30 µl of DNA:Lipofectamine™ 2000 complexes to each well containing cells and medium.

7. Mix gently by tapping the plate.

8. Incubate cells at 37°C in a CO_2 incubator.

Day 3-4:

1. 24 to 48 hours after transfection, measure luciferase levels using the TWO-Step Luciferase Assay System following BPS Bioscience's protocol.

Validation Data

A. Effect of FOXO3 on FOXO reporter activity in HEK293 cells.

- This assay should include "Background Luminescence" and "Transfected" conditions.

Day 1:

1. Seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of cell culture growth medium. Leave a few empty wells as "Background Luminescence" control.

2. Incubate cells overnight at 37°C in a CO_2 incubator.

Day 2:

1. Transfect cells with 1 µl of Component A combined with 0.5 µl Component C or Component D per well ("Transfected" wells), following the procedure described above.

Day 3-4:

1. ~24 hours or ~48 hours post-transfection, dilute 100 x Firefly Luciferase Reagent Substrate (Component B) with Firefly Luciferase Reagent Buffer (Component A) (50 µl/well).

2. Add 50 µl of Firefly Luciferase reagent per well and rock at RT for ~15 minutes.

3. Measure firefly luminescence using a luminometer.

4. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C) (50 µl/well).

5. Add 50 µl of Renilla Luciferase reagent per well, rock at RT for ~1 minute and measure Renilla luminescence.

6. To obtain the normalized luciferase activity for the FOXO reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from the FOXO reporter to Renilla luminescence from the control Renilla luciferase vector.

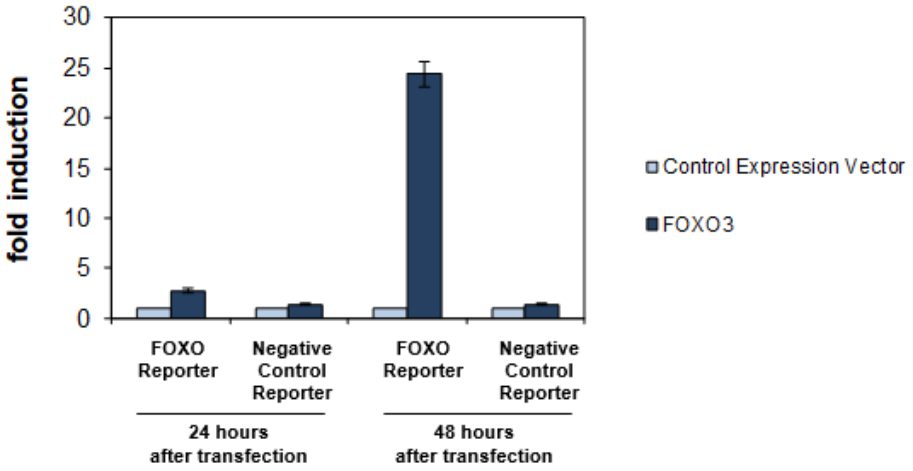

Figure 1. FOXO3 induced expression of the FOXO reporter in HEK293 cells.
HEK293 cells were transfected with FOXO reporter plasmids (Component A) and FOXO3 (Component C) or the Negative Control Reporter (Component D). Luciferase activity was measured with the TWO-Step Luciferase (Firefly & Renilla) Assay System. The results are shown as fold induction of normalized reporter activity by FOXO3 (ratio of normalized reporter activity in the presence of FOXO3 to that in the presence of the negative control expression vector).

B. Effect of PI3K inhibitors on FOXO reporter activity in HEK293 cells.

- This assay should include "Background Luminescence" and "Test Compound" wells.

Day 1:

1. Seed HEK293 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of cell culture growth medium. Leave a few empty wells as "Background Luminescence" control.

2. Incubate cells overnight at 37°C in a CO_2 incubator.

Day 2:

1. Transfect cells with 1 µl of Component A with 0.5 µl of Component C or Component D per well following the procedure described above.

2. Incubate for 6 hours at 37°C in a CO_2 incubator.

3. Prepare the test compound of interest in fresh growth medium at the desired testing concentrations (50 µl/well).

4. ~6 hours post-transfection, carefully remove the media and add 50 µl of test compound in fresh growth medium to the "Test Compound" wells.

5. Add 50 µl of growth medium to the cell-free control wells ("Background Luminescence" control wells).

6. Incubate cells overnight at 37°C in a CO_2 incubator.

Day 3:

1. ~24 hours later, dilute 100 x Firefly Luciferase Reagent Substrate (Component B) with Firefly Luciferase Reagent Buffer (Component A) (50 µl/well).

2. Add 50 µl of Firefly Luciferase reagent per well and rock at RT for ~15 minutes.

3. Measure firefly luminescence using a luminometer.

4. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C) (50 µl/well).

5. Add 50 µl of Renilla Luciferase reagent per well, rock at RT for ~1 minute and measure Renilla luminescence.

6. To obtain the normalized luciferase activity for the FOXO reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from the FOXO reporter to Renilla luminescence from the control Renilla luciferase vector.

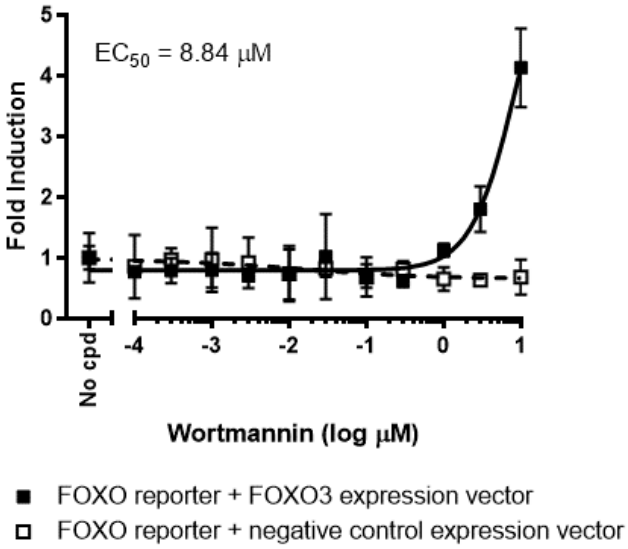

Figure 2. Wortmannin up-regulates FOXO3-induced FOXO reporter activity in HEK293 cells. HEK293 cells were transfected with FOXO reporter plasmids (Component A) and FOXO3 (Component C) or the Negative Control Reporter (Component D) and treated with increasing concentrations of wortmannin. Luciferase activity was measured with the TWO-Step Luciferase (Firefly & Renilla) Assay System. The results are shown as fold induction of normalized reporter activity by FOXO3 (ratio of normalized reporter activity in the presence of FOXO3 to that in the presence of the negative control expression vector). The results are shown as fold induction of FOXO3-induced reporter activity by Wortmannin.

Data shown is 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

Essaghir A., *et al.*, 2009 *J. Biol. Chem.* 284(16):10334-10342
Hennessy B.T., *et al.*, 2005 *Nat. Rev. Drug Discov.* 4(12):988-1004.

Related Products

Products	*Catalog #*	*Size*
PI3 kinase (p110α/p85α)	40620	20 µg
PI3 kinase (p110β/p85α)	40622	20 µg
PI3 kinase (p110δ/p85α)	40628	20 µg
CRE/CREB Reporter Assay Kit	60611	500 reactions
TCF/LEF Reporter Kit (WNT)	60500	500 reactions

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