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Data Sheet
STAT3 Reporter Kit
(STAT3 Signaling Pathway)
Catalog #: 79730

Background

STAT3 (Signal Transducer and Activator of Transcription 3) is a transcription factor which is activated through phosphorylation at Tyrosine 705 in response to various cytokines and growth factors. The phosphorylated STAT3 forms homodimers or heterodimers with STAT1 and the dimers translocated to the nucleus where DNA binding/promoter induction occurs. STAT3 plays an important role in cellular processes such as cell cycle progression and apoptosis.

Description

The STAT3 Reporter kit is designed for monitoring the activity of the STAT3 signaling pathway in cultured cells. The kit contains transfection-ready STAT3 luciferase reporter vector. This reporter contains a firefly luciferase gene under the control of STAT3-responsive element located upstream of a minimal promoter. The STAT3 reporter is premixed with constitutively expressing *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 a negative control. The non-inducible luciferase vector contains the firefly luciferase gene under the control of a minimal promoter, without any additional response elements. The negative control is critical to determining pathway-specific effects and background luciferase activity.

Applications

- Monitor STAT3 pathway activity.
- Screen activators or inhibitors of the STAT3 signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the STAT3 pathway.

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Components

Component	Specification	Amount	Storage
Reporter (Component A)	STAT3 luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C

These vectors are ready-to-use for transient transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen #11668027). However, other transfection reagents work equally well.
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Dual Luciferase (Firefly-Renilla) Assay System (BPS Bioscience #60683): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.
- Luminometer

Generalized 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 condition 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.

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

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a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of following combinations:

- **1 µl of Reporter** (component A); in this experiment, the control transfection is **1 µl of Negative Control Reporter** (component B).
- **1 µl of Reporter** (component A) + experimental vector expressing gene of interest; in this experiment, the control transfections are: **1 µl of Reporte**r (component A) + negative control expression vector, **1 µl of Negative Control Reporter** (component B) + experimental vector expressing gene of interest, and **1 µl of Negative Control Reporter** (component B) + negative control expression vector.
- **1 µl of Reporter** (component A) + specific siRNA; in this experiment, the control transfections are: **1 µl of Reporte**r (component A) + negative control siRNA, **1 µl of Negative Control Reporter** (component B) + specific siRNA, and **1 µl of Negative Control Reporter** (component B) + negative control siRNA.

Note: we recommend setting up at least triplicate assays for each condition, and preparing transfection cocktail for multiple wells.

b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature. Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a CO_2 incubator. After ~24 hours of transfection, treat cells with test activators/inhibitors for additional 6 to 24 hours. Perform the Dual Luciferase Assay System following the protocol on the BPS data sheet (BPS Bioscience #60683).

Sample protocol to determine the dose response of HEK293 cells transfected with STAT3 reporter to IL-6

Additional materials required in this experiment setup

- IL-6 (BPS Bioscience, #90196)
- Thaw Medium 1 (BPS Bioscience #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01)

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- Assay medium: Thaw Medium 1 (BPS Bioscience #60187)
- Dual Luciferase (Firefly-Renilla) Assay System (BPS Bioscience #60683)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate in 100 µl growth medium. Incubate cells at 37°C in a CO_2 incubator for overnight.

2. The next day, transfect 1 µl STAT3 luciferase reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, dilute STAT3 in assay medium and replace cell medium in the stimulated wells with 50 µl diluted IL-6.
Change medium in the unstimulated control wells to 50 µl of assay medium; add 50 µl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

4. Incubate cells at 37°C in a CO_2 incubator for ~ 24 hours.

5. Perform dual luciferase assay using BPS Dual Luciferase (Firefly-Renilla) Assay System (BPS Bioscience #60683): Dilute 100x Firefly Luciferase Reagent Substrate (Component B) into Firefly Luciferase Reagent Buffer (Component A). Add 50 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate (Component D) into Renilla Luciferase Reagent Buffer (Component C). Add 50 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

7. To obtain the normalized luciferase activity for STAT3 reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from the STAT3 reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

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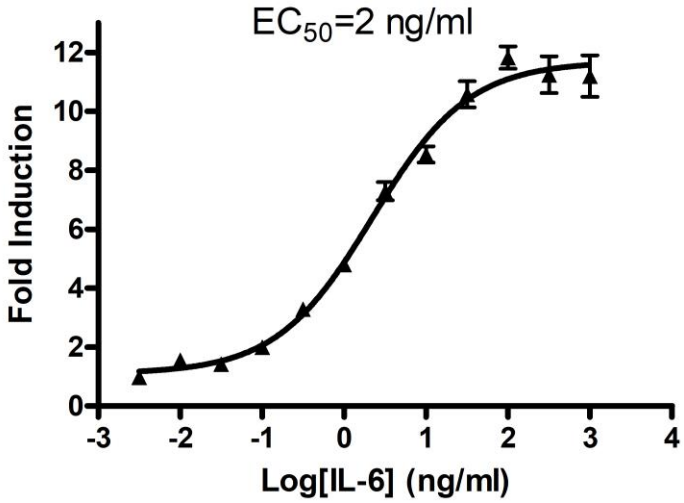

Figure 1. Dose response of STAT3 reporter activity to IL-6 in HEK293
The results are shown as fold induction of normalized STAT3 luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without STAT3 treatment.
The EC50 of IL-6 is ~ 2 ng/ml.

References
1. Tian S., *et al., Blood.* 1994; **84(6):**1760-1764..
2. Zhong, Z., *et al., Science*. 1994; **264(5155):**95-98.

Related products

Product	**Cat. #**	**Size**
Dual Luciferase (Firefly-Renilla) Assay System	60683-1	10 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-2	100 mL
Dual Luciferase (Firefly-Renilla) Assay System	60683-3	1 L
STAT3, GST-tag	75003	20 µg
ISRE Reporter Kit (JAK/STAT Signaling Pathway)	60613	500 rxns.
ISRE Reporter – HEK293 Cell Line (JAK pathway)	60510	2 vials
GAS Reporter (Luc) – HeLa Cell Line (IFNγ/JAK/STAT1 Pathway)	79041	2 vials

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