RapidReporter® pRR-High-STAT3

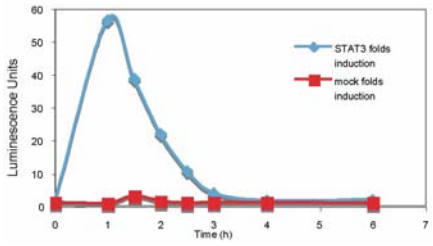
Catalog No: 33013, 33014

Background:
STAT (signal transducers and activators of transcription) transcription factors were discovered fourteen years ago as mediators of interferon-induced gene expression. STAT proteins are latent transcription factors that are activated by phosphorylation via tyrosine kinases. Over 35 different extracellular polypeptides activate JAK associated receptors, leading to phosphorylation of JAKs and the subsequent phosphorylation of STAT proteins. In most cases, STAT activation is transient. Inactivation of STAT proteins is carried out by several mechanisms, including dephosphorylation of STAT proteins in the nucleus and degradation through the ubiquitin-proteosome pathway.

Nucleotide	Vector Site
(none)	Promoter
8-63	STAT3 Tandem Response Element
348-1561	Gaussia luciferase gene (luc+)
2087	ColE1-derived plasmid replication origin
2849-3709	beta-lactamase gene (Ampr)
3861-3896	Synthetic (upstream) poly(A) signal

pRR-REV: 5'-GTTCCCTGCTCTCTGTCG-3'
pRR-FWD: 5'-TCATTACATCTGTGTGTGTT-3'

STAT3 Response Element Sequence: TGCTTCCCGAACGT



Activation of STAT3 in mammalian cells

Prepare the RapidReporter® plasmid DNA, 1X Gaussia Lysis Buffer and Gaussia Assay Buffer with Substrate as outlined in the RapidReporter manual.

DAY 1: Plating Cells
Plating HeLa cells at 1.7 x 10^6 cells/100 mm plate.
Note**:** This amount of cells should yield 50-80% confluence on the day of transfection. Lower confluence is required to allow enough surface area for growth during the experiment period.

1. Carefully aspirate the media from a confluent 100 mm plate and briefly wash twice with 10 mL of PBS
2. Add 1 ml trypsin and incubate for 5 minutes at 37°C in a humidified atmosphere containing 5% CO_2.
3. Resuspend the cells with 9 ml of cell growth media and transfer to a sterile 50 ml conical tube.
4. Count the cells using a hemacytometer.
5. Dilute the cells to the correct density, plate 1.7 x 10^6 cells in 10 ml total media on 100 mm plates and incubate at 37°C in a humidified atmosphere containing 5% CO_2 overnight.

DAY 2: Transient Transfection
1. Ensure that the cells from Day 1 are the correct cell density.
2. For each 100 mm plate of cells to be transfected add 34 µl FuGENE 6 Reagent to 550 µl of Opti-MEM® I Reduced Serum Medium or serum-free culture media being careful not to touch the sidewall of the microcentrifuge tube.
3. Tap to mix and incubate for 5 minutes at room temperature.
4. Add 6-11 µg of pRR vector DNA (or sterile water to mock transfected cells) to the diluted FuGENE 6 mix.

5. Tap to mix and incubate for 15 minutes at room temperature to allow DNA/transfection reagent c
Note: Do not allow undiluted FuGENE 6 Reagent to come into contact with plastic surfaces other than pipette tips. Once the FuGENE 6 Reagent is diluted, combine it with the diluted DNA (from Step 1) within 45 minutes.
6. Tap to mix the transfection mix again and add the entire mixture to each plate. Swirl and return the plate(s) to the 37°C incubator containing 5% CO2 (there is no need to remove the media or wash the cells). Allow the cells to transfect for at least 4-5 hours before re-plating.

Replating onto 96-well plates 5 hours after transfection:
We recommend Costar 9102 strip-well plates
1. Carefully aspirate the media from the 100 mm plate and wash twice with 10 ml of sterile PBS.
Note: Add the PBS slowly to the side of the plate to minimize disturbing the cells.
2. Add 1 ml trypsin and incubate for 5 minutes at 37°C in a humidified atmosphere containing 5% CO2.
3. Resuspend the trypsinized cells with 9 ml of cell growth media and transfer to a 50 ml conical tube for counting.
4. Count cells using a hemacytometer to determine cells per ml of media. Dilute cells with cell growth media to 30,000 cells per 190 µl of media (depending on the cell line used, optimal plating density will vary).
5. Transfer the transiently transfected cells to a sterile plastic reservoir and add 190 µl to each well using a muliti-channel pipette.
6. Return 96-well plates to the 37°C incubator (humidified atmosphere containing 5% CO2) for overnight (at least 16 hours).

Day 3: Stimulation, Lysis and Measurement:
1. Prepare a 20X stock of Oncostatin M (2 µg/ml) in cell growth media.
2. Add the stimulator to a sterile plastic reservoir
3. Using a multichannel pipette add 10 µl of Oncostatin M (2 µg/ml) to each well designated for stimulation. This corresponds to a 100 ng/ml final concentration)
Note: Negative pRR transfected control wells do not receive any transcriptional stimulator; however, the same stimulation treatment is done with mock transfected cells.
4. The plate is returned to the 37°C incubator for the required amount of time.

Lysis and Measurement:
Using 1X Lysis Buffer and Assay Buffer prepared as described in the RapidReporter manual.
1. After the desired times post-stimulation, for example: 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, or 15 hours, remove media from 96-well culture plate and add 20 µl of 1X Lysis Buffer perwell.
2. Incubate at room temperature for at least 30 minutes (minimum lysis times and volumes may vary between cell types, 3 hours is the maximum time form cell lysis). Samples may be stored at room temperature for several hours or at < -20°C for longer-term storage if desired.
Note: We have noted that compared to other luciferases, Gaussia luciferase-containing lysates are more resistant to prolonged storage and freeze-thaw cycles.
3. Measure flash luminescence using a luminometer set to inject 60 µl of 1X Assay Buffer (with substrate) per well (ideally followed by a 1 second shaking) and an immediate read. If an injecting luminometer is not available, manually add 1X Assay Buffer (with substrate) at 60 µl per well by columns using a multichannel pipette, and immediately read in a luminometer.
Note: We use the Tecan Genios luminometer set at the following measurement parameters:
a. Set the gain to 150 and the temperature to maintain at 27°C.
b. Set the machine to measure luminescence and allow for stabilization prior to reading plates (read without plate prior to adding substrate/assay buffer).
c. Choose the correct plate map and read by column (i.e. A1, B1…).

Data Analysis:
Calculate the fold of induction of stimulated versus unstimulated cells from transfected and mock-transfected cells. Graph fold of induction versus time.

Storage
Luciferase Assays provide 100 or 1000 reactions of 5X Lysis and 1X Assay Buffers and Gaussia Substrate. Vector kits provide 10 µg of either empty or pre-made vector. Assays sold with vector contain the appropriate vector, 100 reactions of Lysis and Assay Buffers and Gaussia Substrate, as well as a positive control vector driven by the EF1a promoter. Store at -20°C, see manual for details. All reagents are guaranteed stable for 6 months when stored properly.