Human/Mouse GSK3A (S21) Phosphorylation ELISA Kit (BA0244)

Product Datasheet | SKU BA0244

Description

A fluorimetric cell-based ELISA kit for the determination of GSK3A(S21) phosphorylation status in whole cells and evaluation of pathway modulation by activators, inhibitors, siRNA etc. Phosphorylated pGSK3A(S21) is measured at λex/em = 530/585 nm and normalised to total cellular protein measured at 360/450 nm in the same well.

Specifications

Detection Method	Cell-Based ELISA (FL530/585 nm, 360/450 nm)
Sample Type	Cells
Species Reactivity	Human, mouse, rat and canine
Assay Time	Assay takes 6.5 hrs; hands-on time 2.5 hrs
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	6 months
Shipping	Gel Pack

Kit Components

Component	Quantity	Storage
Stock Wash Buffer	25 mL	-20°C
Blocking Buffer	25 mL	-20°C
Protein Stain	6 mL	-20°C
Dye Reagent	120 µL	-20°C
Ab1	10 µL	-20°C
Ab2 (gM-HRP)	10 µL	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0244	100 Assays

Assay Procedure

Note: This is a sample protocol. Protocols are batch/lot specific - always follow the instructions supplied with your kit.

1. Prepare 1x Wash Buffer by diluting Stock Wash Buffer 40-fold with dH2O (e.g. 10 mL Stock Wash Buffer + 390 mL dH2O). Reserve 6 mL for the Detection step. Assay samples in triplicate or more, and include a Protein Blank (no cells) and a Sample Blank (cells with no Ab1, only Ab2).
2. Culture and treat cells. Seed 100 µL of 1-3×10^4 adherent cells (or 4-10×10^4 suspension cells) per well of a black 96-well culture plate; add 100 µL cell-free media into three wells for the Protein Blank. Incubate overnight at 37°C, then treat cells as desired.
3. Fix cells (caution: formaldehyde is toxic; use a chemical hood and appropriate protection). For adherent cells, replace media with 100 µL 4% formaldehyde; for suspension cells, add 100 µL 8% formaldehyde to the pellet. Incubate 20 min at room temperature, then wash twice with 200 µL 1× Wash Buffer.
4. Quench. Add 100 µL Quench Buffer (2.2 mL 3% H2O2 + 8.8 mL 1× Wash Buffer) per well; incubate 20 min at room temperature, then wash three times with 200 µL 1× Wash Buffer.
5. Block. Add 100 µL Blocking Buffer per well; incubate 1 hr at room temperature.

6. Add Primary Antibody (Ab1). Prepare 55 µL of Ab1 Mixture per well by mixing Ab1 with Blocking Buffer at 1:1000. Add 50 µL Blocking Buffer to Sample Blank wells and 50 µL Ab1 Mixture to Sample wells; incubate 90 min at room temperature (or overnight at 2-8°C) with gentle shaking, then wash three times.
7. Add Secondary Antibody (Ab2). Prepare 55 µL Ab2 Mixture per well by mixing Ab2 with Blocking Buffer at 1:1000; add 50 µL to all wells; incubate 90 min at room temperature with gentle shaking.
8. Detection. Wash four times with 200 µL 1× Wash Buffer. Prepare HRP Substrate (60 µL Dye Reagent + 6 mL 1× Wash Buffer + 6 µL 3% H_2O_2), add 50 µL per well and incubate 30 min at room temperature in the dark. Add 50 µL Protein Stain per well and incubate a further 5 min in the dark.
9. Read the plate at λex/em = 530/585 nm for the phosphorylated target and at λex/em = 360/450 nm for total protein.

Calculation

Calculate the mean pTarget fluorescence at 530/585 nm for the Sample Blank (“No Ab1” wells, F_pTarget.Blank) and Sample wells (F_pTarget.Sample), and the mean protein fluorescence at 360/450 nm for the Protein Blank (F_Protein.Blank) and Sample wells (F_Protein.Sample). ΔF_pTarget = F_pTarget.Sample – F_pTarget.Blank; ΔF_Protein = F_Protein.Sample – F_Protein.Blank. Normalised pTarget = (ΔF_pTarget/ΔF_Protein) / (ΔF_pTarget/ΔF_Protein)O, where (ΔF_pTarget/ΔF_Protein)O is the control reference value (e.g. time zero in kinetic studies or untreated wells in drug potency studies).

Notes

- To avoid cross-contamination, change pipette tips between additions of each reagent or sample; use of a multi-channel pipette and separate reservoirs is recommended.
- It is recommended that samples be assayed in triplicate or more.
- Two blanks in triplicate are required: a Protein Blank (no cells) and a Sample Blank (cells with no Ab1, only Ab2), used to determine background fluorescence for total protein and pGSK3A(S21) respectively.
- The cell number used depends on the cell line and the phosphorylation level of the target protein.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.