Human SHP2 (Y542) Phosphorylation ELISA Kit (BA0255)

Product Datasheet | SKU BA0255

Description

Fluorimetric cell-based assay for the quantitative determination of SHP2(Y542) phosphorylation status in whole human cells, with normalisation to total cellular protein in the same well.

Specifications

Detection Method	Cell-Based ELISA (fluorimetric, FL 530/585 nm and 360/450 nm)
Sample Type	Cells
Species Reactivity	Human
Assay Time	6.5 hours total (hands-on time 2.5 hours)
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
BA0255	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 1x Wash Buffer 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) into each well of a black 96-well culture plate, add 100 µL cell-free media to three wells for the Protein Blank, and incubate overnight at 37°C. Treat cells as desired.
3. Fix cells with freshly prepared formaldehyde solution (4 wt% for adherent cells; 8 wt% for suspension cells) for 20 min at room temperature. Remove and wash twice with 200 µL 1x Wash Buffer (3 min gentle shaking per wash).
4. Quench with 100 µL Quench Buffer (2.2 mL of 3% H2O2 + 8.8 mL 1x Wash Buffer) for 20 min at room temperature, then wash three times with 200 µL 1x Wash Buffer.
5. Block with 100 µL Blocking Buffer for 1 hr at room temperature.

6. Add primary antibody: prepare Ab1 Mixture (Ab1 in 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: prepare Ab2 Mixture (Ab2 in Blocking Buffer at 1:1000). Add 50 µL to all wells; incubate 90 min at room temperature with gentle shaking, then wash four times.
8. Detection: prepare HRP Substrate (60 µL Dye Reagent + 6 mL 1x Wash Buffer + 6 µL 3% H2O2). Add 50 µL to each well and incubate 30 min at room temperature in the dark.
9. Add 50 µL Protein Stain to each well and incubate a further 5 min at room temperature in the dark.
10. Read the plate at λex/em = 530/585 nm for pSHP2(Y542) and at λex/em = 360/450 nm for total protein.

Calculation

Calculate the mean pTarget fluorescence at 530/585 nm for the Sample Blank (FpTarget.Blank) and Sample wells (FpTarget.Sample), and the mean protein fluorescence at 360/450 nm for the Protein Blank (FProtein.Blank) and Sample wells (FProtein.Sample). Determine specific values: ΔFpTarget = FpTarget.Sample – FpTarget.Blank and ΔFProtein = FProtein.Sample – FProtein.Blank. Normalised pTarget = (ΔFpTarget / ΔFProtein) / (ΔFpTarget / ΔFProtein)o, where the denominator is the control reference value (e.g. time zero in kinetic studies or untreated wells in drug potency studies).

Notes

- The cell number to be used depends on the cell line and the phosphorylation level of the target protein.
- To avoid cross-contamination, change pipette tips between additions of each reagent or sample; a multi-channel pipette and separate reservoirs are recommended.
- Formaldehyde is toxic; prepare formaldehyde solutions with caution in a chemical hood while wearing appropriate gloves and eye protection.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.