Human/Mouse RPS6 (S235/236) Phosphorylation ELISA (BA0254)

Product Datasheet | SKU BA0254

Description

A fluorimetric cell-based ELISA for RPS6(S235/236) phosphorylation status in whole cells. Phosphorylated pRPS6(S235/236) is detected with a specific primary antibody and HRP-conjugated secondary antibody using a fluorogenic substrate (530/585 nm) and normalised to total protein (360/450 nm) in the same well.

Specifications

Detection Method	Cell-based ELISA, fluorimetric (λex/em = 530/585 nm and 360/450 nm)
Sample Type	Cultured cells (whole cells)
Species Reactivity	Human, mouse and rat
Assay Time	6.5 hours (hands-on time 2.5 hours)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	-20°C
Shelf Life	About 6 months after receipt
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
BA0254	100 Assays

Assay Procedure

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

1. Preparation: change pipette tips between additions of each reagent or sample; a multi-channel pipette is recommended, using separate reservoirs for each reagent. Prepare 1x Wash Buffer by diluting Stock Wash Buffer as directed (LKB1: 15-fold; RB and RPS6: 40-fold, reserving 6 mL 1x Wash Buffer for the Detection step). Assay samples in triplicate or more, with two blanks in triplicate: a Protein Blank (no cells) and a Sample Blank (cells with no Ab1, only Ab2).
2. A. 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 into three wells for the Protein Blank. Incubate overnight at 37°C. Treat cells as desired.
3. Fix cells (caution: formaldehyde is toxic; use a chemical hood, gloves and eye protection). Adherent cells: prepare 4 wt% formaldehyde (1.3 mL 37% formaldehyde + 10.7 mL 1x Wash Buffer) and replace media with 100 µL per well. Suspension cells: prepare 8 wt% formaldehyde (2.6 mL 37% formaldehyde + 9.4 mL

1x Wash Buffer), centrifuge (500 × g, 15 min, 4°C), remove media and add 100 µL to the pellet. Incubate 20 min at room temperature (fixed plates may be stored sealed up to 2 weeks at 2-8°C).

4. Wash cells twice with 200 µL 1x Wash Buffer (gentle shaking, 3 min each). Prepare Quench Buffer (2.2 mL 3% H2O2 + 8.8 mL 1x Wash Buffer), add 100 µL per well and incubate 20 min at room temperature. Wash 3 times with 200 µL 1x Wash Buffer. Add 100 µL Blocking Buffer and incubate 1 hr at room temperature.
5. B. Add primary antibody (Ab1). Prepare 55 µL Ab1 Mixture per well (Ab1 in Blocking Buffer, 1:1000). Remove Blocking Buffer; 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. Wash 3 times with 200 µL 1x Wash Buffer.
6. C. Add secondary antibody (Ab2). Prepare 55 µL Ab2 Mixture per well (Ab2 in Blocking Buffer, 1:1000). Add 50 µL to all wells and incubate 90 min at room temperature with gentle shaking.
7. D. Detection. Wash 4 times with 200 µL 1x Wash Buffer. Prepare HRP Substrate immediately before use (LKB1: add 6 µL 3% H2O2 to the 6 mL HRP Substrate; RB and RPS6: mix 60 µL Dye Reagent with 6 mL 1x Wash Buffer and 6 µL 3% H2O2). Add 50 µL to each well and incubate 30 min at room temperature in the dark. Add 50 µL Protein Stain to each well and incubate a further 5 min in the dark.
8. 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 phospho-target fluorescence at 530/585 nm for the Sample Blank ('No Ab1' wells, 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). Specific fluorescence values: ΔFpTarget = FpTarget.Sample – FpTarget.Blank; ΔFProtein = FProtein.Sample – FProtein.Blank. Normalized pTarget = (ΔFpTarget / ΔFProtein) / (ΔFpTarget / ΔFProtein)o, where (ΔFpTarget / ΔFProtein)o is the control reference value (e.g. time zero in kinetic studies or untreated wells in drug potency studies).

Notes

- Normal precautions for laboratory reagents should be exercised while using these reagents.
- Reagents are for research use only. Refer to the MSDS for detailed safety information.