



Human/Mouse RB (T821/826) Phosphorylation ELISA Kit (BA0253)

Product Datasheet | SKU BA0253

Description

A fluorimetric cell-based ELISA for RB(T821/826) phosphorylation status in whole cells. Phosphorylated pRB(T821/826) 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 ($\lambda_{\text{ex/em}}$ = 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
BA0253	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 $1-3 \times 10^4$ adherent cells (or $4-10 \times 10^4$ 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 ($500 \times g$, 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 $2-8^{\circ}\text{C}$).
4. Wash cells twice with 200 μL 1x Wash Buffer (gentle shaking, 3 min each). Prepare Quench Buffer (2.2 mL 3% H_2O_2 + 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 $2-8^{\circ}\text{C}$) 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% H_2O_2 to the 6 mL HRP Substrate; RB and RPS6: mix 60 μL Dye Reagent with 6 mL 1x Wash Buffer and 6 μL 3% H_2O_2). 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 $\lambda_{\text{ex/em}} = 530/585 \text{ nm}$ for the phosphorylated target and at $\lambda_{\text{ex/em}} = 360/450 \text{ nm}$ 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: $\Delta\text{FpTarget} = \text{FpTarget.Sample} - \text{FpTarget.Blank}$; $\Delta\text{FProtein} = \text{FProtein.Sample} - \text{FProtein.Blank}$. Normalized pTarget = $(\Delta\text{FpTarget} / \Delta\text{FProtein}) / (\Delta\text{FpTarget} / \Delta\text{FProtein})_0$, where $(\Delta\text{FpTarget} / \Delta\text{FProtein})_0$ 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.

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Technical specifications are based on manufacturer documentation and may be updated. Protocols shown are summaries - always follow the instructions supplied with your kit/lot.