



Human/Mouse 4EBP1 (S65) Phosphorylation ELISA Kit (BA0233)

Product Datasheet | SKU BA0233

Description

A fluorimetric cell-based assay for 4EBP1(S65) phosphorylation status. Cells grown in a 96-well plate are fixed and permeabilised; p4EBP1(S65) is detected using a specific primary antibody, an HRP-conjugated secondary antibody and a fluorogenic substrate (read at 530/585 nm), while a protein stain measures total protein (read at 360/450 nm) for normalisation.

Specifications

Detection Method	Fluorimetric cell-based ELISA ($\lambda_{ex/em}$ = 530/585 nm for p4EBP1(S65) and 360/450 nm for total protein)
Detection Range	Cell-based phosphorylation status assay (normalised to total cellular protein)
Sample Type	Cells (cultured whole cells)
Species Reactivity	Human (S65), mouse/rat (S64)
Assay Time	6.5 hours (hands-on time 2.5 hours)
Kit Size	100 Assays
Equipment Required	Microplate reader
Storage	This kit is shipped on ice. Upon delivery, store all reagents at -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 μ L	-20°C
Ab1	10 μ L	-20°C
Ab2 (gM-HRP)	10 μ L	-20°C

Ordering Information

Assay Genie Cat. No.	Pack Size
BA0233	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; 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, no Ab1).
2. Culture and treat cells: seed 100 μ L of $1-3 \times 10^4$ adherent cells (or $4-10 \times 10^4$ suspension cells) per well of a black 96-well culture plate; add 100 μ L cell-free media to three wells as Protein Blank. Incubate overnight at 37°C, then treat cells as desired.

3. Fix cells with 4% (adherent) or 8% (suspension) formaldehyde solution for 20 min at room temperature (handle formaldehyde with caution in a chemical hood). Wash twice with 200 μ L 1x Wash Buffer with gentle shaking (3 min each).
4. Add 100 μ L Quench Buffer (2.2 mL 3% H₂O₂ + 8.8 mL 1x Wash Buffer) per well; incubate 20 min at room temperature. Wash three times with 200 μ L 1x Wash Buffer.
5. Add 100 μ L Blocking Buffer; incubate 1 hr at room temperature.
6. Add primary antibody: prepare 55 μ L Ab1 Mixture per well (Ab1 : Blocking Buffer, 1:1000). Add 50 μ L Blocking Buffer to Sample Blank wells and 50 μ L Ab1 Mixture to Sample wells. Incubate 90 min at room temperature (or overnight at 2-8°C) with gentle shaking. Wash three times.
7. Add secondary antibody: prepare 55 μ L Ab2 Mixture per well (Ab2 : Blocking Buffer, 1:1000). Add 50 μ L to all wells; incubate 90 min at room temperature with gentle shaking. Wash four times.
8. Detection: prepare HRP Substrate (60 μ L Dye Reagent + 6 mL 1x Wash Buffer + 6 μ L 3% H₂O₂). Add 50 μ L per well; incubate 30 min at room temperature in the dark.
9. Add 50 μ L Protein Stain per well; incubate a further 5 min in the dark. Read at $\lambda_{ex/em}$ = 530/585 nm for p4EBP1(S65) and 360/450 nm for total protein.

Calculation

Calculate mean p4EBP1(S65) fluorescence at 530/585 nm for the Sample Blank (F_pTarget.Blank) and Sample wells (F_pTarget.Sample), and mean protein fluorescence at 360/450 nm for the Protein Blank (F_Protein.Blank) and Sample wells (F_Protein.Sample). $\Delta F_{pTarget} = F_{pTarget.Sample} - F_{pTarget.Blank}$; $\Delta F_{Protein} = F_{Protein.Sample} - F_{Protein.Blank}$. Normalised pTarget = $(\Delta F_{pTarget} / \Delta F_{Protein}) / (\Delta F_{pTarget} / \Delta F_{Protein})_0$, where the subscript 0 is the control reference value (e.g. time zero or untreated wells).

Notes

- To avoid cross-contamination, change pipette tips between additions of each reagent or sample and use separate reservoirs for each reagent. A multi-channel pipette is recommended.
- The cell number to be used depends on the cell line and the phosphorylation level of the target protein.
- Fixed-cell plates can be sealed and stored for up to 2 weeks at 2-8°C.
- 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.