# Human/Mouse AKT1 (S473) Phosphorylation ELISA Kit (BA0235)

Product Datasheet | SKU BA0235

## Description

A fluorimetric cell-based assay for AKT1(S473) phosphorylation status. Cells grown in a 96-well plate are fixed and permeabilised; pAKT1(S473) 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

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|---|---|
| **Detection Method** | Fluorimetric cell-based ELISA (λex/em = 530/585 nm for pAKT1(S473) 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, rat and mouse |
| **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 (10x) | 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 |
|---|---|
| BA0235 | 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 20-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 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 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 µL 1x Wash Buffer with gentle shaking (3 min each).
4. Add 100 µL 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 µL 1x Wash Buffer.
5. Add 100 µL Blocking Buffer; incubate 1 hr at room temperature.
6. Add primary antibody: prepare 55 µL Ab1 Mixture per well (Ab1 : Blocking Buffer, 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. Wash three times.
7. Add secondary antibody: prepare 55 µL Ab2 Mixture per well (Ab2 : Blocking Buffer, 1:1000). Add 50 µL to all wells; incubate 90 min at room temperature with gentle shaking. Wash four times.
8. Detection: prepare HRP Substrate (60 µL Dye Reagent + 6 mL 1x Wash Buffer + 6 µL 3% H2O2). Add 50 µL per well; incubate 30 min at room temperature in the dark.
9. Add 50 µL Protein Stain per well; incubate a further 5 min in the dark. Read at λex/em = 530/585 nm for pAKT1(S473) and 360/450 nm for total protein.

## Calculation

Calculate mean pAKT1(S473) 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). 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)_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.