Version 10-07/25

User's Manual

PhosphoHiLA™ Human Phospho-AKT1 (Ser473) Detection Kit

REF **PHILA-BY170**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

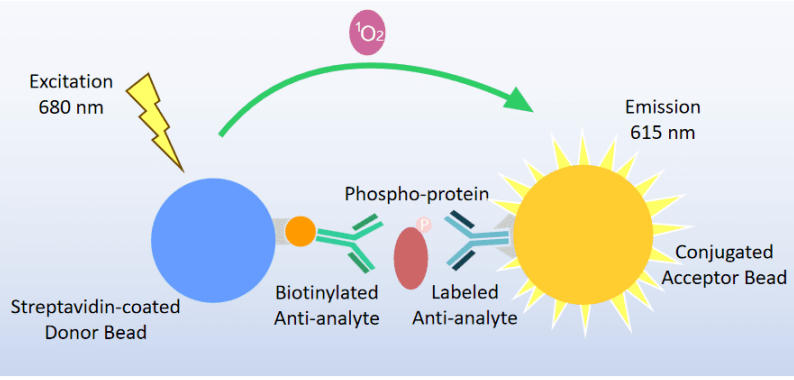
The Human Phospho-AKT1 (Ser473) Detection Kit can be used to measure levels of AKT1 phosphorylated at residue Ser473 in cellular lysates.

General Description

Phospho-AKT1 (Ser473) indicates full AKT1 activation via mTORC2-mediated phosphorylation at the hydrophobic motif. This primes AKT1 to phosphorylate substrates like cytoplasmic ME2 at Ser9, blocking mitochondrial translocation and enhancing glycolysis. Phosphorylated ME2 scaffolds glycolytic enzymes (PFKL, PKM2), shifting metabolism to Warburg effect. Hyperactivation drives tumor growth in liver and breast cancers, with p-AKT1(Ser473) serving as a biomarker for PI3K pathway activity and therapeutic resistance.

Principles of Testing

The PhosphoHiLA™ assay kits enable the rapid and sensitive detection of phosphorylated or total cellular proteins. The kit utilizes bead-based Homogeneous luminescence Technology. The Acceptor Bead is coated with a proprietary agent to specifically immobilize the assay specific antibody which is labeled with a tag. The Donor Bead is coated with streptavidin to capture the biotinylated antibody.
In the presence of a target protein, the two antibodies bring the Donor and Acceptor Beads in close proximity. This enables the generation of a signal upon illumination of Donor Beads by the Homogeneous ImmunoLuminescence Assay abled plate reader. The amount of light emission is directly proportional to the amount of target protein present in the sample.
This assay system performs well in the presence of extraneous antibodies, such as antibody biotherapeutics, and can be used to screen such reagents.
This assay eliminates the need for laborious techniques, such as Western blotting or conventional ELISA.
It is a homogeneous assay with no washing steps, minimal handling, short incubation times, enhanced signal-to-noise windows, better well-to-well reproducibility (lower CV%), and robotic operation if desired.



Reagents And Materials Provided

1. Lysis Buffer (5×), 1 × 12 mL
2. Supplement (only in kits with Lysis Buffer B or C (5×)), 1 × 1.5 mL
3. Activation Buffer, 1 × 0.8 mL
4. Reaction Buffer 1, 1 × 1.5 mL

5. Reaction Buffer 2, 1 × 1.5 mL
6. Dilution Buffer, 1 × 3 mL
7. Acceptor Beads (2mg/mL in PBS plus 0.03% Proclin-300), 1 × 0.06 mL
8. Streptavidin Donor Beads (2mg/mL in PBS plus 0.03% Proclin-300), 1 × 0.06 mL
9. Positive Control Lysate, 1 × Lyophilized tube

Storage

Unopened kit: Store at 4°C. DO NOT freeze the kit. The Reaction Buffer contains antibodies and freeze/thaw cycles can lead to a loss of activity.

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