Version 16-07/25

User's Manual

PhosphoHiLA™ Human and Mouse Total AKT1 Detection Kit

REF **PHILA-BY220**

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RUO

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

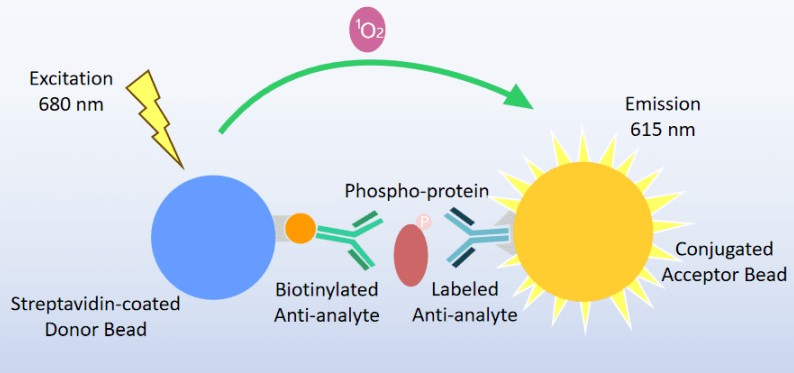
The Human and Mouse Total AKT1 Detection Kit is a sandwich immunoassay for quantitative detection of Akt1 (phosphorylated and non-phosphorylated) in cellular lysates.

General Description

Total AKT1 (PKBα) is a serine/threonine kinase central to PI3K signaling, regulating survival, proliferation, and metabolism. Activated by PIP3 binding and phosphorylation (Thr308/Ser473), it inhibits pro-apoptotic proteins (e.g., BAD) and enhances glucose uptake. Overexpressed in >50% of NSCLC tumors, AKT1 correlates with advanced TNM stage, lymph node metastasis, and poor 3-year survival (13.21% vs. 37.84% in low-AKT1 patients). Isoform-specific inhibitors (e.g., ipatasertib) target hyperactivation but struggle with toxicity due to AKT1"s metabolic roles.

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.