

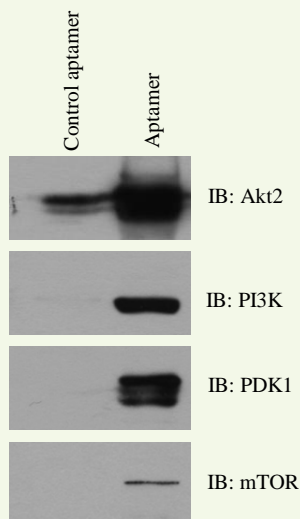
Anti-Akt2 aptamer, Dual Magnetic AP/Co-AP Kit™



Introduction

When a protein is expressed at low levels and is difficult to detect with western blot analysis, aptoprecipitation (AP, Aptamer based protein pull down method) may be the method of choice. An aptoprecipitating reagent has to be specific in order to avoid precipitation of unwanted protein. Furthermore, sufficient affinity is required to pull down the protein and it has to withstand stringent washing steps. AptSci Akt2 aptamer molecule is a specific affinity ligand and has been proven well suited for pull down experiments of Akt2 proteins. Most commonly encountered problems with IP approach is interference from antibody heavy and light chains that may co-migrate with relevant bands, masking important results. However aptamer as an oligonucleotide will not contribute to protein/peptide background that can interfere with subsequent analysis.

AptSci has developed proprietary protein pull down method using target protein-specific aptamers. The Akt2 AP/Co-AP Kit makes it possible to control physiologically relevant protein-protein interactions as well as reducing non-specific bindings by the addition of polymer with charge. The aptamer-coupled magnetic bead has low nonspecific binding characteristic and enables convenient magnetic isolation of protein targets and reusable magnetic beads. Mild elution condition enables isolation of non-denatured proteins which can be used for further study.



Co-Aptoprecipitation (Co-AP)

Figure 1 shows that the Akt2 protein and Akt2 interacting partners were precipitated from MCF7 cell extract using Akt2 aptamer-coupled magnetic bead. An intense Akt2 band was clearly obtained by using the Akt2 aptamer, while no Akt2 band was detected when precipitating with control aptamer. Akt2 interacting proteins such as PI3K, PDK1 and mTOR were also identified in aptamer based Co-AP assay. Akt2 aptamers were highly specific to Akt2 protein and Akt2 aptamer-coupled magnetic bead efficiently precipitates Akt2 as well as Akt2 interacting proteins from a complex protein mix. These results indicated that Akt2 aptamer based Co-AP assay can be a useful tool for the identification of physiologically relevant Akt2 protein-protein interactions.

Product Information

- **Product name:** Anti-Akt2 aptamer, Dual Magnetic AP/Co-AP Kit™
- **Content:** Magnetic agarose conjugated anti- Akt2 aptamer molecule and all buffers required to perform small scale AP
- **Catalog number:** Akt2 -2154DDM
- **Protein source for generation of aptamer:** Recombinant protein produced in mammalian cells
- **Specificity:** Anti- Akt2 aptamer binds to human Akt2. Cross reactivity with other species has not been tested.
- **MW:** 17.3 kDa
- **Conjugation yield:** > 90% as determined by spectrometer analysis.
- **Tested applications:** Co-aptoprecipitation.
- **Form:** As 25% slurry in 20% ethanol containing 0.04% (w/v) sodium azide.
- **Storage:** At +4°C.
- **Shipping:** At ambient temperature.
- **Stability:** There is no decrease in performance of the kit after storage for 6 months at ambient temperature.

Fig. 1. Co-Aptoprecipitation of Akt2 and their interacting proteins from MCF7 cells using the AptSci Akt2 AP/Co-AP Kit. MCF7 cell lysates (1mg/lane) were incubated with Akt2 aptamers (500pmol)-coupled magnetic bead. After washing the beads, the bound protein complex was eluted with high-pH elution buffer. The eluate was separated by 4-12% SDS-PAGE and blotted onto a PVDF membrane. Western blot was probed with specific antibodies (anti Akt Ab, anti PI3K Ab, anti PDK1 Ab, and anti mTOR Ab). Control aptamer: Aptamer (Reverse complement sequence of Akt2 aptamer)-coupled magnetic bead was used as a control.

LIMITATIONS

Warranty: AptSci AP Kit™ products are warranted to meet stated product specifications and to confirm to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to AptSci's instructions. AptSci's sole liability is limited to replacement of the product or refund of the purchase price. AP Kit™ products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. AP Kit™ products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from AptSci.