Anti-Akt2 aptamer, Direct Magnetic 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 aptamer-coupled magnetic bead included in the kit 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.

Result of Aptoprecipitation (AP)

As shown in Figure 1, Western blot analysis revealed that Akt2 aptamer precipitates Akt2 with high specificity, while no Akt2 was observed when precipitating with anti-Akt2 antibody. Akt2 band was also obtained by high-pH elution buffer, while no Akt2 was observed when eluting with low-pH elution buffer included in antibody IP Kit. These results indicate that Akt2 aptamer is highly specific to Akt2 protein and Akt2 aptamer-coupled magnetic bead efficiently precipitates Akt2 from a protein complex.

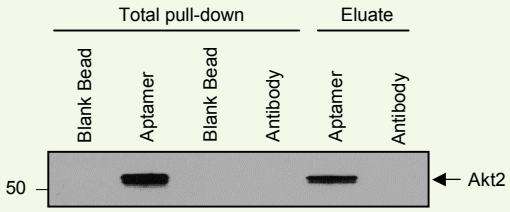

Fig. 1. Aptoprecipitation of Akt2 protein from MCF7 cells using the AptSci direct Akt2 AP Kit. MCF7 cell lysates (1mg/lane) were incubated with either Akt2 aptamer (50pmol)-coupled magnetic bead or anti-Akt2 antibody (50pmol)-coupled magnetic bead (Dynabead M270). Conjugation was performed according to product instructions. After washing the beads, the bound protein was eluted in either boiling SDS loading buffer, high-pH elution buffer (aptamer) or low-pH elution buffer (antibody). After neutralization, the eluate was loaded on a SDS-PAGE (4-15% gradient gel) and blotted onto a PVDF membrane. Western blot was probed with anti Akt2 Ab

Product Information

- **Product name**: Anti-Akt2 aptamer, Direct Magnetic AP Kit
- **Catalog number**: Akt2-2154DM
- **Content**: Magnetic agarose conjugated Akt2 aptamer molecule and all buffers required to perform small scale AP
- **Form**: As 25% slurry in 20% ethanol containing 0.04% (w/v) sodium azide.
- **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**: ~18 kDa
- **Conjugation yield**: > 90% as determined by spectrometer analysis.
- **Tested applications**: Aptoprecipitation.
- **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.

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