

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

amsbio

Introduction

When a protein is expressed at low levels and is difficult to detect with western blot analysis, apoprecipitation (AP, Aptamer based protein pull down method) may be the method of choice. An apoprecipitating 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 INSR (Insulin receptor) aptamer molecule is a specific affinity ligand and has been proven well suited for pull down experiments of INSR 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 INSR 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.

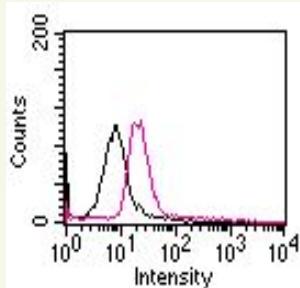


Fig. 1. Flow cytometry histograms showing the binding of representative INSR aptamer against the target Rat-1/INSR cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated INSR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal (Black histogram).

Results Apoprecipitation (AP)

As shown in figure 2, an intense INSR proteins were precipitated from Rat-1/INSR cell extract using INSR aptamer-coupled magnetic bead. An intense IR bands (INSR α and INSR β) were clearly obtained by using the INSR aptamer, while no INSR bands were detected when precipitating with control aptamer.

In summary, INSR aptamers were highly specific to INSR receptor (INSR α and INSR β) and INSR aptamer-coupled magnetic bead efficiently precipitates INSR from a complex protein mix.

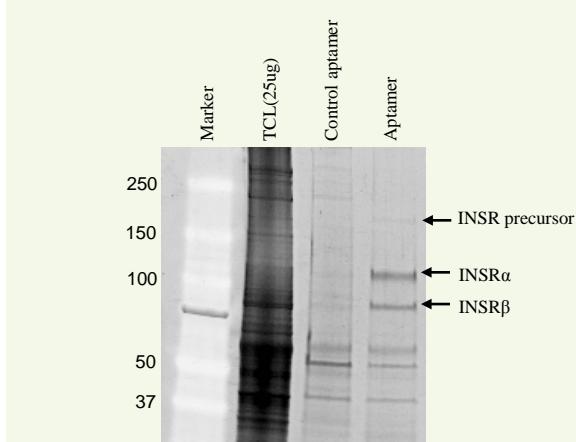


Fig. 2. Apoprecipitation of INSR protein from Rat-1/INSR cells using the AptSci INSR AP/Co-AP Kit. Rat-1/INSR cell lysates (1mg/lane) were incubated with INSR aptamers (70pmol)-coupled magnetic beads. The bound protein was eluted with boiling SDS-loading buffer and separated with SDS-PAGE (4-15% gradient gel). The gel was directly stained with SYPRO ruby. TCL: Total cell lysate. Control aptamer: Aptamer (Reverse complement sequence of IR aptamer)-coupled magnetic beads is used as a control

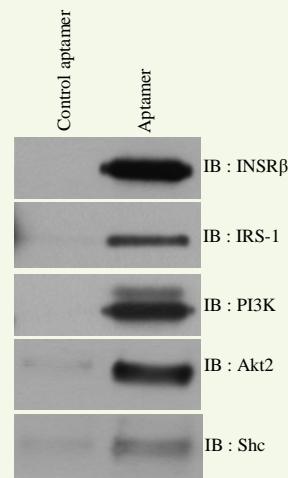


Fig. 3. Co-Apoprecipitation of INSR and their interacting proteins from Rat-1/INSR cells using the AptSci INSR AP/Co-AP Kit. Subconfluent Rat-1/INSR cell culture was starved overnight and stimulated with 100 nM insulin. Rat-1/INSR cell lysates (1mg/lane) were incubated with INSR aptamer (500pmol)-coupled magnetic bead. The bound protein was eluted with high-pH elution buffer. The samples were separated by SDS-PAGE and blotted onto a PVDF membrane. The membrane was probed with specific antibodies (anti INSR β Ab, anti IRS-1 Ab, anti PI3K Ab, anti Akt2 Ab, and anti Shc Ab). TCL: Total cell lysate. Control aptamer: Aptamer (Reverse complement sequence of IR aptamer)-coupled magnetic bead was used as a control

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Results Co-Aptoprecipitation (Co-AP)

Figure 3 shows that the INSR protein and INSR interacting partners were precipitated from Rat-1/INSR cell extract using INSR aptamer-coupled magnetic bead. An intense INSR β band was clearly obtained by using the INSR aptamer, while no INSR β band was detected when precipitating with control aptamer. INSR interacting proteins such as IRS-1, PI3K, Akt2 and Shc were also identified in aptamer based Co-AP assay. INSR aptamers were highly specific to INSR protein and INSR aptamer-coupled magnetic bead efficiently precipitates INSR as well as INSR interacting proteins from a complex protein mix. These results indicated that INSR aptamer based Co-AP assay can be a useful tool for the identification of physiologically relevant INSR protein-protein interactions.

Product Information

- **Product name:** Anti-INSR aptamer, Dual Magnetic AP/Co-AP Kit™
- **Content:** Magnetic agarose conjugated anti-INSR aptamer molecule and all buffers required to perform small scale AP
- **Catalog number:** INSR -1652DDM
- **Protein source for generation of aptamer:** Recombinant protein produced in mammalian cells.
- **Specificity:** Anti-INSR aptamer binds to human INSR. Cross reactivity with other species has not been tested.
- **MW:** 14.4 kDa
- **Conjugation yield:** > 90% as determined by spectrometer analysis.
- **Tested applications:** FACS and Aoptoprecipitation.
- **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.

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.



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