

Anti-INSR 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 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 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.

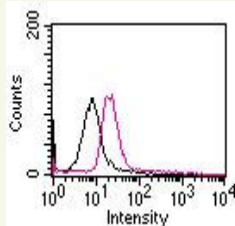


Fig. 1. Flow cytometry histograms showing the binding of representative INSR aptamer against the target Rat-1/INSR cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated INSR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal (Black histogram).

Result of Aptomprecipitation (AP)

Figure 2 shows that the INSR proteins were precipitated from Rat-1/INSR cell extract using INSR aptamer-coupled magnetic bead. It should be noted that many proteins which are not related with INSR were detected in IP using antibodies. Moreover, anti-INSR antibody precipitates small amounts of INSR receptor. On the other hand, much less non-specific binding of protein was observed in AP using aptamer. An intense INSR bands (INSR α and INSR β) were obtained by high-pH elution buffer, while a relatively weak INSR was detected with non-specific binding of protein when eluting with low-pH elution buffer included in antibody IP Kit (upper image in Figure 2).

As shown in Figure 2 (lower image), western blot analysis revealed that INSR aptamer precipitates INSR receptor with high specificity, while only small amounts of INSR receptor was observed when precipitating with anti-INSR antibody.

In summary, INSR aptamer-coupled magnetic bead efficiently precipitates INSR from a protein complex.

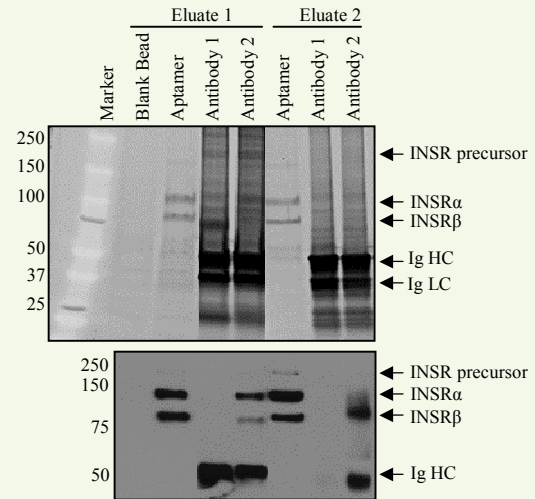


Fig. 2. Aptomprecipitation of INSR protein from Rat-1/INSR cells using the AptSci Direct INSR AP Kit. Rat-1/INSR cells lysates (1mg/lane) were incubated with either INSR aptamer (50pmol)-coupled magnetic bead or anti-INSR antibodies (50pmol)-coupled magnetic bead (Dynabead M270). The bound protein was eluted in either SDS-sample buffer (eluate 1), high-pH elution buffer (eluate 2, aptamer) or low-pH elution buffer (eluate 2, antibodies) and separated by SDS-PAGE (4-15% gradient gel). The gel was directly stained with SYPRO ruby (upper image). Samples were also blotted onto a PVDF membrane and Western blot was probed with specific antibodies (lower image).

Product Information

- **Product name:** Anti-INSR aptamer, Direct Magnetic AP Kit
- **Catalog number:** INSR-1652DM
- **Content:** Magnetic agarose conjugated INSR 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-INSR aptamer binds to human INSR. Cross reactivity with other species has not been tested.
- **MW:** ~15 kDa
- **Conjugation yield:** > 90% as determined by spectrometer analysis.
- **Tested applications:** FACS and Aptomprecipitation.
- **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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LIMITATIONS

Warranty: AptSci AptoPrep™ 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. AptoPrep™ products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. AptoPrep™ products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from AptSci.