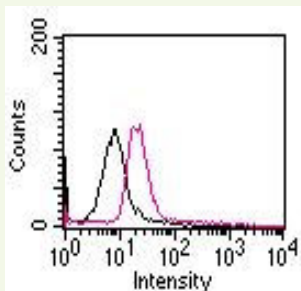


## Anti-INSR aptamer, Indirect Magnetic AP Kit

### 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 biotinylated aptamer has low nonspecific binding characteristic and streptavidin magnetic beads enable convenient magnetic isolation of protein targets. Mild elution condition enables isolation of non-denatured proteins which can be used for further study.

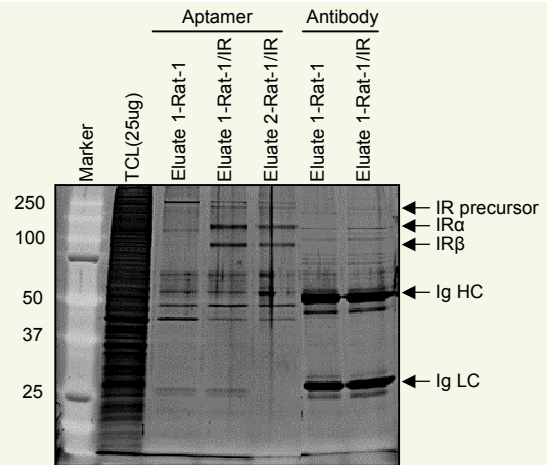


**Fig. 1. Flow cytometry histograms showing the binding of representative INSR aptamer against the target Rat-1/INSR cells.** Approximately  $1 \times 10^6$  cells were washed and incubated with FITC-conjugated INSR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal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

### Result of Apto-precipitation (AP)

Figure 2 shows that the INSR proteins were precipitated from Rat-1/INSR cell extract using the biotinylated INSR aptamer. An intense INSR bands (INSR $\alpha$  and INSR $\beta$ ) were clearly obtained by using the biotinylated IR aptamer, while a relatively weak INSR band was detected when precipitating with anti-INSR antibody. An intense INSR bands (INSR $\alpha$  and INSR $\beta$ ) were also obtained by high-pH elution buffer. Both INSR $\alpha$  and INSR $\beta$  protein were precipitated from Rat-1/ INSR cells but not from Rat-1 cells when precipitating with either INSR aptamer or antibody.

In summary, the biotinylated INSR aptamer molecule efficiently precipitates INSR from a complex protein mix, while anti-INSR antibodies precipitates extraordinarily small amounts of INSR.



**Fig. 2. Apto-precipitation of INSR protein from Rat-1/ INSR cells using the AptSci Indirect INSR AP Kit.** Rat-1/INSR cell lysates (1mg/lane) were incubated with either biotinylated INSR aptamers (40pmol) or anti-INSR antibody (40pmol). The mixed solution was further incubated with either Streptavidin magnetic beads (aptamer) or Protein A bead. The bound protein was eluted in either SDS-sample buffer (eluate 1) or high-pH elution buffer (eluate 2) and separated by SDS-PAGE (4-15% gradient gel). The gel was directly stained with SYPRO ruby. TCL: Total cell lysate.

### Product Information

- **Product name:** Anti-INSR aptamer, Indirect Magnetic AP Kit
- **Catalog number:** INSR-1652IM
- **Content:** Biotinylated anti-INSR aptamer, Streptavidin Magnetic Bead and all buffers required to perform small scale AP
- **Form:** Biotinylated aptamer is supplied in a dried form and Streptavidin Magnetic Bead is supplied in PBS pH7.4, containing 0.01% Tween-20 and 0.09% NaN<sub>3</sub>.
- **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 Apto-precipitation.
- **Storage:** At 2-8°C.
- **Shipping:** At cooling condition.
- **Stability:** There is no decrease in performance of the kit after storage for 6 months at ambient temperature.



[www.aptsci.com](http://www.aptsci.com), Aptamer Sciences Inc.  
 Postech Biotech Center, San31, Hyoja-Dong, Pohang,  
 Gyeongbuk 790-784, South Korea. TEL +82-54-279-8691  
 FAX +82-54-279-8245. E-mail: aptamer@aptsci.com.

### 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.