

Anti-EphA2 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 EphA2 aptamer molecule is a specific affinity ligand and has been proven well suited for pull down experiments of EphA2 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.

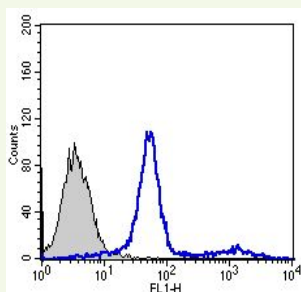


Fig. 1. Flow cytometry histograms showing the binding of representative EphA2 aptamer against the target A549 cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated EphA2 aptamer (Blue histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Results Aptoprecipitation (AP)

Figure 2 shows that the EphA2 proteins were precipitated from A549 cell extract using EphA2 aptamer-coupled magnetic bead. An intense EphA2 was observed by using EphA2 aptamer, while there was no EphA2 detected when precipitating with blank bead, as shown in figure 2.

In summary, EphA2 aptamers were highly specific to EphA2 receptor and EphA2 aptamer-coupled magnetic bead efficiently precipitates EphA2 from a complex protein mix.

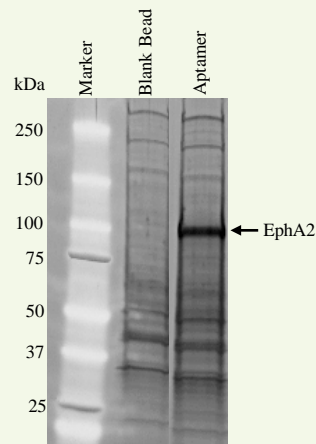


Fig. 2. Aptoprecipitation of EphA2 protein from A549 cells using the AptSci Direct EphA2 AP Kit. A549 cell lysates (1mg/lane) were incubated with EphA2 aptamer (150pmol)-coupled magnetic bead. After washing the beads the bound protein was eluted with boiling SDS sample buffer and separated by SDS-PAGE (4-15% gradient gel). The gel was directly stained with SYPRO ruby.

Product Information

- **Product name:** Anti-EphA2 aptamer, Direct Magnetic AP Kit™
- **Content:** Magnetic agarose conjugated EphA2 aptamer molecule and all buffers required to perform small scale AP
- **Catalog number:** EphA2-2176DM
- **Protein source for generation of aptamer:** Recombinant protein produced in mammalian cells.
- **Specificity:** Anti-EphA2 aptamer binds to human EphA2. Cross reactivity with other species has not been tested.
- **MW:** 25.1 kDa
- **Conjugation yield:** > 90% as determined by spectrometer analysis.
- **Tested applications:** FACS and 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.

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.