

Anti-EGFR aptamer, Dual Magnetic AP/Co-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 EGFR aptamer molecule is a specific affinity ligand and has been proven well suited for pull down experiments of EGFR 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 EGFR 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.

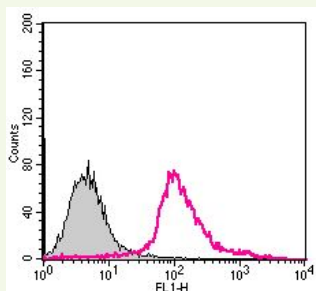


Fig. 1. Flow cytometry histograms showing the binding of representative EGFR aptamer against the target A431 cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated EGFR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Results Aptoprecipitation (AP)

An intense EGFR band was clearly obtained by using the EGFR aptamer without sacrificing specific binding of EGFR, while no EGFR bands were detected when precipitating with control aptamer, as shown in figure 2. Furthermore, much less non-specific protein-binding was observed in AP. On the other hand, EGFR protein was not clearly detected from IP with antibody. These results indicated that EGFR aptamers were highly specific to EGFR receptor and EGFR aptamer-coupled magnetic bead efficiently precipitates EGFR from a complex protein mix.

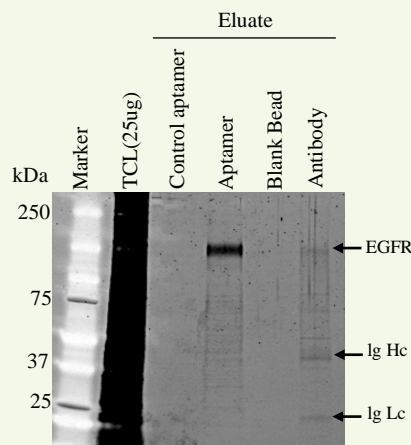


Fig. 2. Aptoprecipitation of EGFR protein from A431 cells using the AptSci EGFR AP/Co-AP Kit. A431 cell lysates (1mg/lane) were incubated with either EGFR aptamer (50pmol)-coupled magnetic bead or anti-EGFR antibody (50pmol)-coupled magnetic bead (Dynabead M270). The bound protein was eluted and separated with SDS-PAGE. The gel was directly stained with SYPRO ruby. TCL: Total cell lysate. Control aptamer: Aptamer (Reverse complement sequence of EGFR aptamer)-coupled magnetic beads is used as a control

Results Co-Aptoprecipitation (Co-AP)

Figure 3 shows that the EGFR protein and EGFR interacting partners were precipitated from A431 cell extract using EGFR aptamer-coupled magnetic bead. EGFR was clearly detected in Co-AP eluted proteins from total proteins bound to bead, while EGFR protein was not clearly detected in Co-IP eluted proteins from total proteins bound to bead. EGFR aptamers were highly specific to EGFR, while anti-EGFR Abs precipitated extraordinarily small amounts of EGFR. EGFR interacting proteins such as PLC- γ 1 and PI3K were also identified in Co-AP assay, while any of EGFR interacting proteins were not clearly detected in Co-IP assay. Although data are not shown here, other EGFR interacting proteins such as Shc and Grb2 were clearly identified in Co-AP assay. Interestingly, PDK1 and Akt2 protein involved in EGFR signal pathway were also identified (data not shown) in Co-AP assay. These results indicated that EGFR aptamer based Co-AP assay can be a useful tool for the identification of physiologically relevant EGFR protein-protein interactions.

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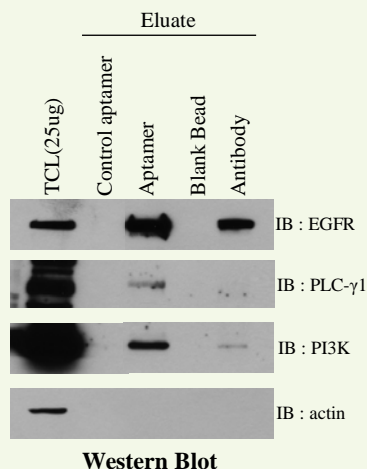


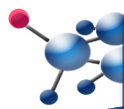
Fig. 3. Co-Aptoprecipitation of EGFR and their interacting proteins from A431 cells using the AptSci EGFR AP/Co-AP Kit. Subconfluent A431 cell culture was starved overnight and stimulated with 100 nM EGF. A431 cell lysates (3mg/lane) were incubated with either EGFR aptamer (500pmol)-coupled magnetic bead or anti-EGFR antibody (100pmol)-coupled magnetic bead (Dynabead M270). The bound protein was eluted and separated with SDS-PAGE and blotted onto a PVDF membrane. The membrane was probed with specific antibodies (anti EGFR Ab, anti PLC- γ 1 Ab, and anti PI3K Ab). TCL: Total cell lysate. Control aptamer: Aptamer (Reverse complement sequence of EGFR aptamer)-coupled magnetic bead was used as a control

Product Information

- **Product name:** Anti-EGFR aptamer, Dual Magnetic AP/Co-AP Kit™
- **Content:** Magnetic agarose conjugated anti-EGFR aptamer molecule and all buffers required to perform small scale AP
- **Catalog number:** EGFR-2369DDM
- **Protein source for generation of aptamer:** Recombinant protein produced in mammalian cells
- **Specificity:** Anti-EGFR aptamer binds to human EGFR. Cross reactivity with other species has not been tested.
- **MW:** 17.3 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 conform 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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