

Anti-EGFR 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 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 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.

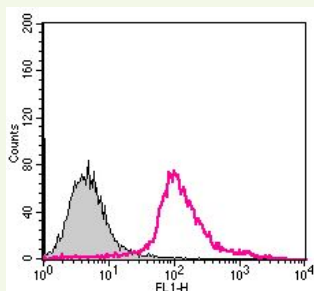


Fig. 1. Flow cytometry histograms showing the binding of representative EGFR aptamer against the target A431 cells. Approximately 1×10^6 cells were washed and incubated with FITC-conjugated EGFR aptamer (Pink histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Results Aptoprecipitation (AP)

Figure 2 shows that the EGFR proteins were precipitated from A431 cell extract using EGFR aptamer-coupled magnetic bead. It should be noted that many proteins which are not related with EGFR were detected in IP using antibodies. Moreover, anti-EGFR antibody precipitates small amounts of EGFR receptor. On the other hand, much less non-specific binding of protein was observed in AP using aptamer. EGFR band was obtained by high pH elution buffer, while a relatively weak EGFR was detected with non-specific binding of protein when eluting with low-pH elution buffer included in antibody IP Kit.

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

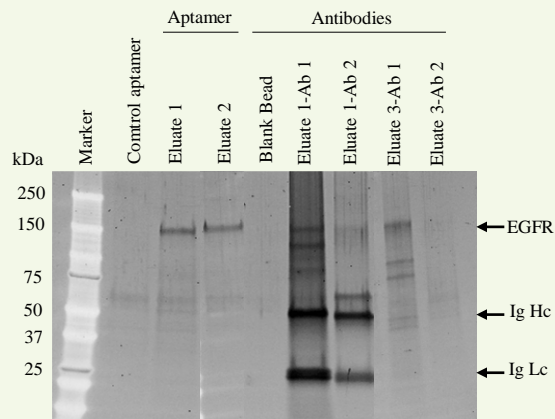


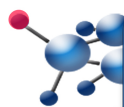
Fig. 2. Aptoprecipitation of EGFR protein from A431 cells using the AptSci Direct EGFR AP Kit. A431 cell lysates (1mg/lane) were incubated with either EGFR aptamer (50pmol)-coupled magnetic bead or anti-EGFR antibody (50pmol)-coupled magnetic bead (Dynabead M270). The bound protein was eluted in either SDS-sample buffer (eluate 1), high-pH elution buffer (eluate 2) or low-pH elution buffer (eluate 3). The samples were separated by SDS-PAGE (4-15% gradient gel) and stained with SYPRO ruby. Control aptamer: Aptamer (Reverse complement sequence of EGFR aptamer)-coupled magnetic beads is used as a control

Product Information

- **Product name:** Anti-EGFR aptamer, Direct Magnetic AP Kit™
- **Content:** Magnetic agarose conjugated EGFR aptamer molecule and all buffers required to perform small scale AP
- **Catalog number:** EGFR-2369DM
- **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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