Anti-ErbB2 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 ErbB2 aptamer molecule is a specific affinity ligand and has been proven well suited for pull down experiments of ErbB2 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.

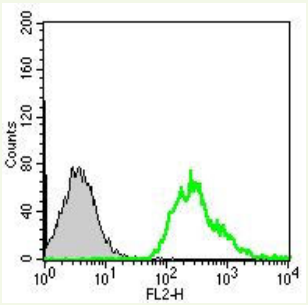

Fig. 1. Flow cytometry histograms showing the binding of representative ErbB2 aptamer against the target MCF7/ErbB2 cells. Approximately 1 × 10^6 cells were washed and incubated with FITC-conjugated ErbB2 aptamer (Green histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Result of Aptoprecipitation (AP)

Figure 2 shows that the ErbB2 proteins were precipitated from MCF7/ErbB2 cell extract using ErbB2 aptamer-coupled magnetic bead. An intense ErbB2 band was clearly obtained by using the ErbB2 aptamer, while there was no ErbB2 detected when precipitating with blank beads, as shown in figure 2.

In summary, ErbB2 aptamers were highly specific to ErbB2 receptor and ErbB2 aptamer-coupled magnetic bead efficiently precipitates ErbB2 from a protein complex.

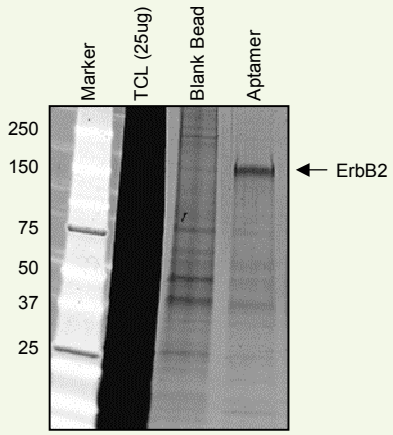

Fig. 2. Aptoprecipitation of ErbB2 protein from MCF7/ ErbB2 cells using the AptSci Direct ErbB2 AP Kit. MCF7/ErbB2 cells lysates (1mg/lane) were incubated with ErbB2 aptamer (100pmol)-coupled magnetic bead. After washing the beads, the bound protein was eluted with SDS-sample buffer and separated with SDS-PAGE (4-15% gradient gel). The gel was directly stained with SYPRO ruby. TCL: Total cell lysate

Product Information

- **Product name**: Anti-ErbB2 aptamer, Direct Magnetic AP Kit
- **Catalog number**: ErbB2-1194DM
- **Content**: Magnetic agarose conjugated ErbB2 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-ErbB2 aptamer binds to human ErbB2. Cross reactivity with other species has not been tested.
- **MW**: ~14 kDa
- **Conjugation yield**: > 90% as determined by spectrometer analysis.
- **Tested applications**: FACS and Aptoprecipitation.
- **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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