Anti-ErbB2 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 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 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.

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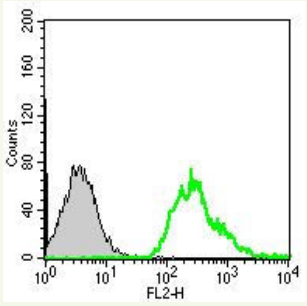

Fig. 1. Flow cytometry histograms showing the binding of representative ErbB2 aptamer against the target MCF7/ErbB2 cells. Approximately 1 × 10^6 cells were washed and incubated with FITC-conjugated ErbB2 aptamer (Green histogram). The untreated cell was used as background fluorescence signal (Gray histogram).

Result of Aptoprecipitation (AP)

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Figure 2 shows that the ErbB2 proteins were precipitated from MCF7/ErbB2 cell extract using the biotinylated ErbB2 aptamer. An intense ErbB2 band was clearly obtained by using the biotinylated ErbB2 aptamer, while a relatively weak ErbB2 band was observed when precipitating with anti-ErbB2 antibody. ErbB2 protein was precipitated from MCF7/ErbB2 cells but not from MCF7 cells when precipitating with either aptamer or antibody.

In summary, the biotinylated ErbB2 aptamer molecule efficiently precipitates ErbB2 from a protein complex.

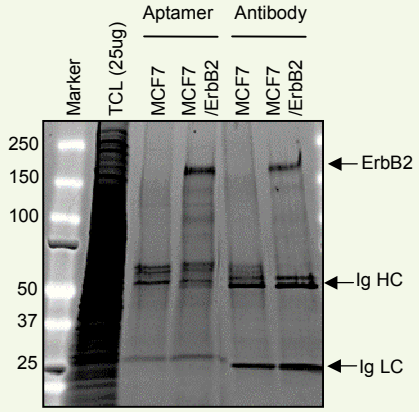

Fig. 2. Aptoprecipitation of ErbB2 protein from Rat-1/ ErbB2 cells using the AptSci Indirect ErbB2 AP Kit. Rat-1/ErbB2 cell lysates (1mg/lane) were incubated with either biotinylated ErbB2 aptamers (40pmol) or anti-ErbB2 antibody (40pmol). The mixed solution was further incubated with either Streptavidin 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-ErbB2 aptamer, Indirect Magnetic AP Kit
- **Catalog number**: ErbB2-1194IM
- **Content**: Biotinylated anti-ErbB2 aptamer, Streptavidin Magnetic Bead and 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% NaN3.
- **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**: ~15 kDa
- **Conjugation yield**: > 90% as determined by spectrometer analysis.
- **Tested applications**: FACS and Aptoprecipitation.
- **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.

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