Anti-Akt2 aptamer, Indirect Magnetic AP Kit (Catalog No. Akt2-2154IM)

Description

Indirect Magnetic AP Kit contains sufficient reagents to perform 40 reactions using biotinylated Akt2 aptamer and streptavidin magnetic beads.

Kit Contents

Reagent	Details
Biotinylated Akt2 Aptamer: Biotinylated anti-human Akt2 aptamer (MW: ~18 kDa) is supplied in a dried form.	**Use.** Used to precipitate Akt2 and Akt2 interacting proteins in cell lysate. **Quantity.** 1 vial (~15 µg)
Streptavidin coated Magnetic Beads, the bead is supplied in PBS pH7.4, containing 0.01% Tween-20 and 0.09% sodium azide.	**Use.** Used as pull-down of aptamer **Quantity.** 1 vial (0.4 mL)
5X APB (Aptoprecipitation buffer, filtered), binding buffer, pH 7.5	**Use.** Used as cell lysis **Quantity.** 1 vial (16 mL)
10X WB (Wash buffer, filtered), pH 7.5	**Use.** Used as washing of the beads **Quantity.** 1 vial (12 mL)
1X EB (High-pH elution buffer, filtered), pH 11.3	**Use.** Used as elution of target proteins **Quantity.** 1 vial (4 mL)
1X NB (Neutralizing buffer, filtered), pH 7.5	**Quantity.** 1 vial (1 mL)
20X S1 solution	**Use.** Used to reduce nonspecific binding of proteins **Quantity.** 1 vial (2 mL)

Reagent and instrument requirements

Magnetic stand
Rotating or Rocking mixer
Protein electrophoresis equipment
Sterile Phosphate-buffered saline (PBS, pH 7.4)
Protease inhibitors without EDTA
biotin-conjugated control aptamer (optional)
DNase I (optional)

Storage/Stability

Store the kit at 2–8˚C upon receipt and when not in use. Kit product is stable at 2–8˚C for at least 1 year.

Procedure

Note: The aptoprecipitation protocol may be performed at 4~8°C to avoid protein complex dissociation and minimize enzymatic activity.

Preparation of Cell Extract

1. Carefully remove culture medium from cells.
2. Wash the cells twice with ice-cold PBS and remove excess PBS from the cell pellet.
3. Lyse cells with ice-cold 1X APB containing protease inhibitors. Incubate on ice for 10 minutes or sonicate on ice briefly.

Note: Prepare cell extract with 10^5 ~ 10^6 cells in 100mm culture dish (approximately 1~2 mg of total cellular extract proteins) per mL of 1X APB. If low Akt2 protein expression is anticipated, use a higher concentration extract by increasing the number of cells per mL.

4. Centrifuge at ~13,000 × g for 10 minutes at 4˚C to pellet the cell debris.
5. Transfer supernatant carefully to a new tube for protein concentration determination and further analysis. The cell extract is now ready for protein aptoprecipitation.

Preparation of Aptamer

1. Dissolve the stock aptamers completely in 400 µl of H_2O. The reconstituted aptamer should be stored at -20°C to 4°C until use.

Note: The concentration of aptamer is 2 pmol/ µl

2. 10 µl of aptamers are diluted in 10 µl of 2X APB and heated for 5 minutes at 95˚C followed by cooled for 20 minutes at RT.

Pull Down

Note: The following protocol is optimized for 10 µl (20 pmol) of biotinylated aptamer and 10 µl of streptavidin (SA) magnetic beads, but this procedure can be scaled up to prepare larger quantities of biotinylated aptamer. The volume of cell extract and the amount of biotinylated aptamer can be determined by the user, if necessary.

Note: As a control for analysis, you can choose the magnetic beads (included in kit) or the control aptamer-magnetic bead (refer to related products).

1. Dilute the lysate solution with APB to 1~2mg/mL.
2. Add prepared aptamer to 1 mL of solubilized supernatant (Combine 0.95 mL cell extract plus 0.05mL 20X S1 solution).

Note: S1 solution can be useful in minimizing nonspecific binding of proteins. If you have weak signal of target protein rather than nonspecific binding of proteins, please use less amount of S1 solution.

3. Incubate the mixture for 2 hours at 4˚C to form protein complex with gentle rotation.

(continued)

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4. Prepare beads during step 3. Wash 10 µl of SA magnetic beads twice with 0.5 mL of 1X WB and collect the beads with a magnetic stand. Remove completely the supernatant and resuspend the beads with 10 µl of 1X APB.
5. Add the reaction mixture of step 3 to SA magnetic beads and incubate the mixture for 10 min at 4˚C
6. Place the tube on a magnetic stand and discard the supernatant.
7. Wash the beads by adding 0.5 mL of 1X WB and gently invert to mix for 1 minute. Collect the beads on a magnetic stand and remove the supernatant.
8. Repeat twice steps 7. In the final step, remove completely the supernatant. The complex is now ready for elution from the beads collected in the bottom of the tube.

Note: If more milder washing is required, WB can be substituted to PBS. Occasionally, the use of 1X WB can release the interesting protein or complex from the beads.

Elution

Note: Two methods are available for elution of the bound complex from the beads.

1. High pH elution

(1) Add 30 µl of 1X EB to the tube and incubate for 15 minutes at room temperature with gentle mixing. It is recommended to elute at 37˚C for a higher elution efficiency.
(2) Place the tube on a magnetic stand and transfer the supernatant to new tube.
(3) Add 3 µl of 1X NB to the supernatant for the neutralization and mix immediately.

Note: The eluted sample can be analyzed by SDS-PAGE gel, Western blot, and downstream protein assay

2. Dnase I elution (optional method)

(1) Add 30µl of reaction buffer and 0.5U~1U of Dnase I to the tube according to the manufacturer, and incubate for 60 minutes at 30~37˚C.
(2) Place the tube on a magnetic stand and transfer supernatant to new tube.

Note: If the desired amount of protein is not obtained, additional elution with Dnase I at least twice more is recommended to ensure that the protein has completely eluted. This method is highly specific to obtain the protein of interest from the beads without protein damage. The eluted sample can be usable for study of SDS-PAGE gel, Western blot, or downstream protein assay.

Alternative Elution: Add SDS-PAGE Sample Buffer to the tube and heat the samples at 95~100˚C in a heating block for 5 minutes. Magnetically separate the beads and save the supernatant-containing target complex.

Sample Preparation for SDS-PAGE Analysis

Note: The eluate obtained by using the methods as described above is now ready for downstream analysis.

1. Resuspend the eluate in SDS-PAGE sample buffer.
2. Heat the sample at 95~100˚C for 5 minutes, then centrifuge and keep the supernatant. You can run them on a SDS-PAGE or freeze the sample.

Related Products

Product	Catalog No.
Control aptamer	CA-2154
Anti-Akt2 aptamer, Direct Magnetic AP Kit	Akt2-2154DM
Anti-Akt2 aptamer, Dual Magnetic AP/Co-AP Kit	Akt2-2154DDM