

Anti-Akt2 aptamer, Direct Magnetic AP Kit (Catalog No. Akt2-2154DM)

Description

Direct Magnetic AP Kit contains sufficient reagents to perform 40 reactions using Akt2 aptamer-magnetic beads.

Kit Contents

Reagent	Details
Akt2 Aptamer-Magnetic Agarose Beads: Anti-human Akt2 aptamer conjugated to magnetic bead is supplied as a 25% slurry in 20% ethanol and 0.04% sodium azide.	Use. Used to precipitate Akt2 and Akt2 interacting proteins in cell lysate Quantity. 1 vial (1.6mL)
Magnetic Agarose Beads, Blank magnetic agarose bead is supplied as a 25% slurry in 20% ethanol and 0.04% sodium azide.	Use. Used as negative control Quantity. 1 vial (1.6 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
Control aptamer-magnetic bead (optional)
DNase I (optional)
5mM MgCl₂ in PBS (optional)

Storage/Stability

Store kit and kit components at 2 – 8°C when not in use. Kit product is stable ambient temperature for at least 1 year. Before using, refer to the product label for the expiration date.

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

- Carefully remove culture medium from cells.
- Wash the cells twice with ice-cold PBS and remove excess PBS from the cell pellet.
- 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 10⁵ ~ 10⁶ 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.

- Centrifuge at ~13,000 × g for 10 minutes at 4°C to pellet the cell debris.
- Transfer supernatant carefully to a new tube for protein concentration determination and further analysis.

Pull Down

Note: The following protocol is optimized for 40 µl of aptamer-magnetic beads (25% slurry), but this procedure can be scaled up to prepare larger quantities of aptamer-magnetic beads. The volume of cell extract and the amount of aptamer-magnetic beads 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).

- Dilute the lysate solution with APB to 1~2 mg/mL.
 - Wash 40 µl of aptamer-magnetic beads twice with 0.5mL of 1X WB and collect the beads with a magnetic stand. Remove the supernatant.
 - Add 1 mL of solubilized supernatant (Combine 0.95 mL of cell extract plus 0.05mL of 20X S1 solution) to the aptamer-magnetic beads.
- 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, use less amount of S1 solution.
- Incubate the mixture for 2 hours at room temperature or overnight at 4°C to capture protein complex with gentle rotation.
 - Place the tube on a magnetic stand and discard the supernatant.
 - Wash the beads by adding 0.5mL of 1X WB and gently invert to mix for 1 minute. Collect the beads on a magnetic stand and remove the supernatant.

(continued)

Anti-Akt2 aptamer, Direct Magnetic AP Kit (Catalog No. Akt2-2154DM)

7. Repeat twice steps 6. 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 complex from the magnetic beads.

Elution

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

1. High pH elution

(1) Add 30µl 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 3µl 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, Mass spectrometry, and downstream protein assay. The aptamer-magnetic beads should be reusable. For details, see in the section reconstitution of aptamer-magnetic bead.

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 interesting protein the beads without protein damage. The eluted sample can be usable for study of SDS-PAGE gel, Western blot or downstream protein assay. However, the aptamer-magnetic beads is not reusable because the aptamer can be degraded by the Dnase I.

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.

Note: The boiled aptamer-magnetic beads can be reusable. For details, see in the section reconstitution of aptamer-magnetic bead.

Reconstitution of aptamer-magnetic bead

Note: This kit has been tested the reusability two times according to the following protocol.

1. Aspirate completely supernatant from the aptamer-magnetic bead and add 50 µl of PBS containing 5mM MgCl₂
2. Heat the aptamer-magnetic beads for 5 minutes at 95°C and cool for 20 minutes at RT.
3. Keep beads in 20% ethanol and store the beads at 2~8°C until use.

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-magnetic bead	CA-2154MB
Anti-Akt2 aptamer, Dual Magnetic AP/Co-AP Kit	Akt2-2154DDM
Anti-Akt2 aptamer, Indirect Magnetic AP Kit	Akt2-2154IM

LIMITATIONS

Warranty: AptSci AptoPrep™ products are warranted to meet stated product specifications and to confirm 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. AptoPrep™ products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. AptoPrep™ products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from AptSci.



UK & Rest of World
184 Milton Park, Abingdon
OX14 4SE, Oxon, UK
Tel: +44 (0) 1235 828 200
Fax: +44 (0) 1235 820 482

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
Tel: +41 (0) 91 604 55 22
Fax: +41 (0) 91 605 17 85

Deutschland
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
Tel: +49 (0) 69 779099
Fax: +49 (0) 69 13376880

North America
23591 El Toro Rd, Suite #180
Lake Forest, CA 92630
Tel: + 1 800 987 0985
Fax: + 1 949 265 7703



amsbio

info@amsbio.com

www.amsbio.com
AMS Biotechnology