Anti-INSR aptamer, Direct Magnetic AP Kit (Catalog No. INSR-1652DM)

Description

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

Kit Contents

Reagent	Details
INSR Aptamer-Magnetic Agarose Beads: Anti-human INSR aptamer conjugated to magnetic bead is supplied as a 25% slurry in 20% ethanol and 0.04% sodium azide.	**Use.** Used to precipitate INSR and INSR 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 $Mgcl_2$ 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

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 INSR 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.

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).

1. Dilute the lysate solution with APB to 1~2 mg/mL.
2. 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.
3. 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.

4. Incubate the mixture for 2 hours at room temperature or overnight at 4°C to capture protein complex with gentle rotation.
5. Place the tube on a magnetic stand and discard the supernatant.
6. 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)

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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 95~100ºC 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 $Mgcl_2$
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℃ 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-1652MB
Anti-INSR aptamer, Dual Magnetic AP/Co-AP Kit	INSR-1652DDM
Anti-INSR aptamer, Indirect Magnetic AP Kit	INSR-1652IM

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