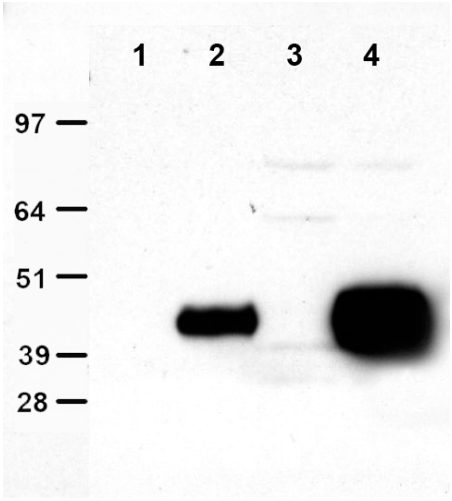
AGAROSE BEADS CONJUGATED WITH ANTI-DDK 4C5 MONOCLONAL ANTIBODY

Catalog Number	TA150038
Product Name	Agarose beads conjugated with 4C5 Anti-DDK mouse monoclonal antibody
Clone ID	4C5
Amount	5mL
Immunogen	DYKDDDDK (the same epitope as Flag)
Specificity	The antibody recognizes DYKDDDDK tag fused to the C-terminus of recombinant proteins
Formulation	TBS (pH7.4) containing 50% Glycerol, 0.02% NaN3
Storage/Stability	Store at -20ºC. Stable for at least 1 year from date of shipment.
Application	The agarose beads conjugated with 4C5 Anti-DDK mouse monoclonal antibody are intended to be used for immunoprecipitation assays. Optimal working dilutions should be determined experimentally by the investigator. Suggested starting dilution 50uL agarose beads conjugated with 4C5 Anti-DDK mouse monoclonal antibody to 5-10ug target protein.
Safety	This product contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation.
Note	This product is for laboratory research use only and is not intended for diagnostic use.
Example of Procedure	1. Prepare 4C5 anti-DDK-conjugated agarose beads by mixing well within the bottle. Also prepare unconjugated agarose beads if the optional step 8 is chosen to perform. 2. Pipette out 50uL unconjugated or conjugated agarose beads and add them to 1.5mL Eppendorf tube. 3. Spin down the agarose beads briefly at 1,500 rpm and remove the supernatant carefully without disturbing the agarose pellet. 4. Wash the agarose beads by applying 1mL RIPA buffer and mixing well. 5. Spin down the agarose beads briefly and remove the supernatant carefully without disturbing the agarose pellet. 6. Repeat steps 4 & 5 three times to clean the agaose beads. 7. Keep the agarose beads on ice if not used immediately. 8. OPTIONAL: Mix the agarose beads unconjugated with any antibody with protein mixture sample (cell lysate expressing target protein and/or negative control lysate) well and incubated for 1 hour

at 4 °C. This step is to pre-clean the sample and avoid the non-specific binding at the following steps.
9. After pre-cleaning, spin down the unconjugated beads-sample mixture for 1 min at 1,500 rpm. Transfer the supernatant carefully to the tube containing the conjugated agarose beads.
10. Mix well and incubate overnight at 4 °C.
11. After overnight incubation, spin down the beads-sample mixture and vacuum out the supernatant.
12. Wash the mixture three times as previously mentioned in steps 4 & 5.
13. After the last washing step, apply 20uL 4XSDS sample buffer to the beads pellet and boil for 10 mins at 95 °C.
14. Spin the agarose beads down for 3-4 mins.
15. Load the supernatant to a SDS-PAGE gel and perform Western blot with appropriate antibodies.

Experimental Data



Immunoprecipitation was performed with HEK293T cell lysates of expressing Entry vector (control, Cat# PS100001) or expressing DDK-tagged PON3 (Cat# LY400339). Anti-DDK 4C5-agarose beads were incubated with control lysate or LY400339 overnight at 4C respectively. After washing three times with lysate buffer, 20uL 4x SDS sample buffer was applied. The samples were boiled at 95C for 10min. The supernatant was loaded to the SDS-PAGE gel after discarded the beads by centrifuging. After transferring to the membrane, the Western blot assay was applied with HRP-conjugated anti-DDK antibody (Cat# TA150030).

Lane 1: control lysate

Lane 2: DDK-tagged PON3 (LY400339)

Lane 3: anti-DDK 4C5 agarose beads (TA150038) 50uL with control lysate

Lane 4: anti-DDK 4C5 agarose beads (TA150038) 50uL with LY400339