MAGNETIC BEADS CONJUGATED WITH ANTI-DDK 4C5 MONOCLONAL ANTIBODY

Catalog Number	TA150042
Product Name	Magnetic beads conjugated with 4C5 Anti-DDK mouse monoclonal antibody
Clone ID	4C5
Amount	1mL
Immunogen	DYKDDDDK (the same epitope as Flag)
Specificity	The antibody recognizes DYKDDDDK tag fused to the C-terminus of recombinant proteins
Formulation	PBS (pH7.4) containing 50% Glycerol, 0.1% BSA, 0.02% NaN3 with final concentration 5mg/mL Beads
Storage/Stability	Store at -20ºC. Stable for at least 1 year from date of shipment.
Application	The magnetic 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 20uL magnetic 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 magnetic beads by mixing well within the bottle. 2. Pipette out 20uL magnetic beads and add them to 1.5mL Eppendorf tube. 3. Apply the tube with beads to a magnetic stand and let it stand for about 30-60 seconds. The beads will be adhesive to the side of the tube. 4. Vacuum out the reagent at the bottom of the tube and avoid touching the magnetic beads on the side. 5. Apply 1mL RIPA buffer to the tube and mix well with the beads. 6. Apply the tube with beads to the magnetic stand. Let it stand for

about 30-60 seconds. Remove the RIPA buffer from the tube.
7. Repeat steps 5 and 6 three times to wash the magnetic beads.
8. Apply the protein mixture sample (cell lysate expressing the target protein, and/or negative control lysate).
9. Mix well the magnetic beads with the sample and incubate overnight at 4°C.
10. After overnight incubation, apply the tube with beads-sample mixture to the magnetic stand. Let it stand for about 30-60 seconds. Vacuum out all the solution and wash the beads with RIPA buffer for 3 times as previously mentioned in steps 5 and 6.
11. After the last washing, apply 20uL 4X SDS sample buffer to the magnetic beads pellet and boil the mixture for 10 mins at 95°C.
12. Spin the magnetic beads down with centrifuge for 3-4 minutes.
13. Load the supernatant to the SDS-PAGE gel and perform the Western blot with appropriate antibody.

Experimental Data

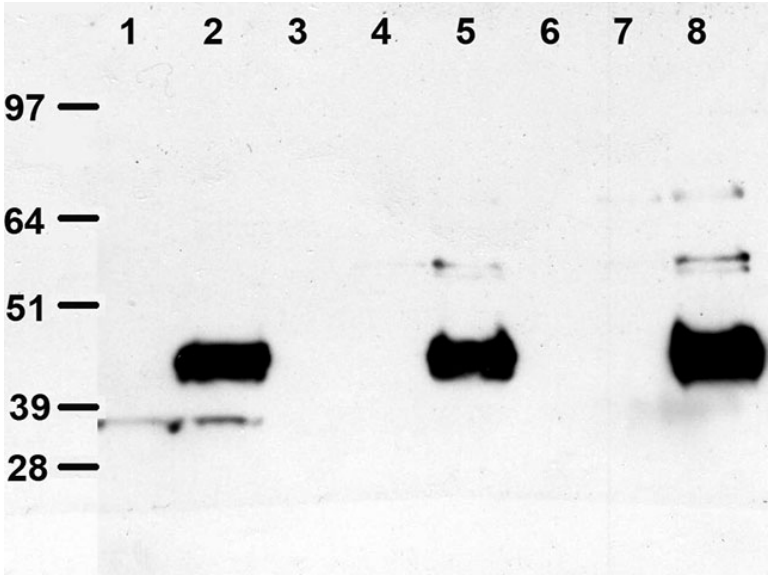

Figure 1. Immunoprecipitation was performed with lysates of HEK293T cells overexpressing a GFP-tagged vector (Cat# PS100010) or DDK-tagged PON3 (Cat# LY400339). Anti-DDK 4C5-magnetic beads were incubated with the GFP lysate or LY400339 overnight at 4C respectively. After washing three times with lysate 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, HRP-conjugated anti DDK antibody (Cat# TA150030) was applied for the Western blot assay.

Lane 1: GFP expressing lysate

Lane 2: DDK-tagged PON3 (LY400339)

Lane 3: Anti-DDK 4C5 magnetic beads (TA150042) only (20uL)

Lane 4: GFP expressing lysate with 20uL anti-DDK 4C5 magnetic beads (TA150042)

Lane 5: LY400339 with 20uL anti-DDK 4C5 magnetic beads (TA150042)

Lane 6: Anti-DDK 4C5 magnetic beads (TA150042) only (50uL)

Lane 7: GFP expressing lysate with 50uL anti-DDK 4C5 magnetic beads (TA150042)

Lane 8: LY400339 with 50uL anti-DDK 4C5 magnetic beads (TA150042)

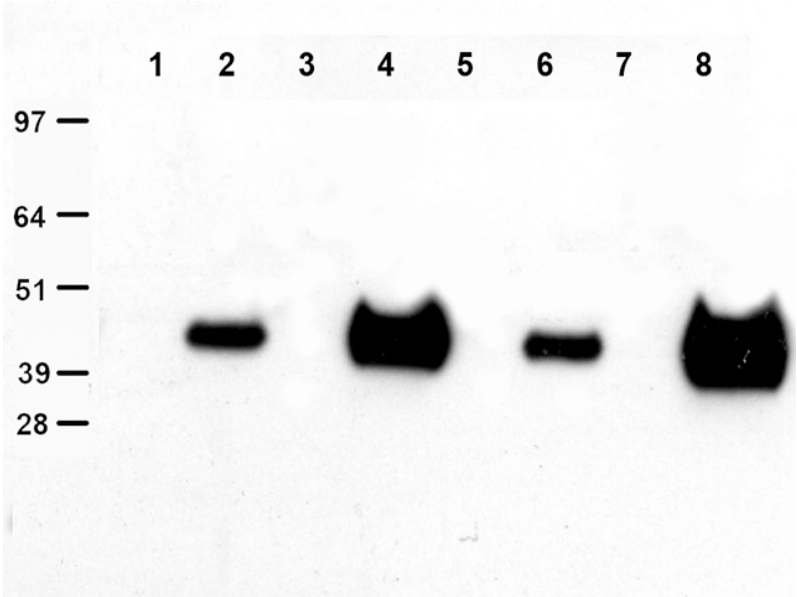

Figure 2. Immunoprecipitation was performed with control lysate of HEK293T cells overexpressing Entry vector (control, Cat# PS100001) or DDK-tagged PON3 (Cat# LY400339). Anti-DDK4C5-magnetic beads were incubated with control lysate or LY400339 overnight at 4C respectively. The same amount of M2-magnetic beads (Sigma, Anti-Flag M2 magnetic beads, M8823) were applied with the equivalent of control lysate or LY400339 at the same time. 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 magnetic beads (TA150042) 20uL with control lysate

Lane 4: Anti-DDK 4C5 magnetic beads (TA150042) 20uL with LY400339

Lane 5: control lysate

Lane 6: LY400339

Lane 7: Anti-Flag M2 magnetic beads 20uL with control lysate

Lane 8: Anti-Flag M2 magnetic beads 20uL with LY400339