Procedure for protein purification (from cell lysate) with DDK/Myc tag antibody conjugated agarose beads (TA150037)

Note: mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform. This is a general guideline for protein purification with antibody conjugated agarose beads, and requires optimization steps for each individual application.

1. Prepare 100µL agarose beads (or mouse IgG conjugated pre-clearing agarose beads, TA120001) and transfer into a 1.5mL microcentrifuge tube. Add 400µL of routine RIPA buffer (or the buffer for the cell lysate) to the beads and vortex gently to mix well.

2. Spin the beads down to the bottom of the tube with bench top centrifuge at 4 °C, remove the supernatant carefully.

3. Wash the agarose beads or pre-clearing agarose beads with RIPA buffer 1.0mL.

4. Repeat step 2 and 3 three times.

5. After the last spin, remove the supernatant and apply DDK tagged protein lysate (0.5-1.0mL) to the beads, mix well, then incubate on the rotator for 1 hour at 4 °C.

a. During this time, prepare 100µL DDK/Myc tag conjugated agarose beads into a 1.5mL microcentrifuge tube. Add 400µL of routine RIPA buffer (or the buffer for cell lysate) to the beads and vortex gently to mix well.

b. Wash the DDK/Myc tag conjugated agarose beads by repeating steps 2 and 3, then place the DDK/Myc tag conjugated agarose beads on ice.

6. Spin the lysate and beads mixture down to the bottom of the tube with bench top centrifuge at 4 °C, transfer the supernatant onto the DDK/Myc tag conjugated agarose beads and mix well.

7. Incubate the mixture on the rotator at 4 °C for 1-2 hours. Then spin the beads down to the bottom of the tube. Remove the supernatant carefully.

8. Add 0.5-1.0mL of RIPA/Washing Buffer to the tube, mix well,

9. Then spin the beads down to the bottom of the tube. Remove the supernatant carefully.

10. Repeat steps 8 and 9 three times.

11. After the last spin and supernatant removal, add 100µL of Elution Buffer (0.1M glycine, pH 2.0-2.3) to the tube, mix well and incubate about 5-10 minutes at room temperature with occasional mixing.

12. Spin the beads down to the bottom of the tube, save the supernatant that contains the eluted protein. 1M Tris buffer (pH 8.5 to 9) can be used to neutralize the low pH.