مؤسسة مستشفى سرطان الأطفال-مصر
Children's Cancer Hospital Foundation - Egypt
57357

Immunoprecipitation

Dynabeads Protein G Immunoprecipitation Kit

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https://www.57357.org/proteomics-unit/

1. Resuspend Dynabeads (DB) in the vial by vortex for 30 sec
2. Transfer 33.3 ul DB solution to new tube
   [Contains 1 mg beads bind maximum to 8 ug of Ab]
3. Place the tube on the magnet to separate the beads from the solution and remove the supernatant
4. In 132 ul of Ab binding and washing buffer (in kit), add up to 8ug of Ab in 200 ul PBS.
5. Mix and add it to DB
6. Incubate for 10 minutes in room temperature (away from the magnet) with occasional tapping
7. Place the tube on the magnet and remove the supernatant

   NB: *Supernatant could be stored for testing.*

8. Add 132 of Ab binding and washing buffer and gentile pipetting (remove unbound Ab and keep the magnet-Ab complex).

   NB: *sample can be stored in this buffer in cold temperature to prevent aggregation.*

9. Place the tube on the magnet and remove the supernatant
10. Add your sample (100 ul to 1ml) and gentile pipette the bead- Ab complex with the sample (Ag)
11. Incubate with rotation for 10-15 minutes at room temperature to allow binding.
12. Place the tube on the magnet and transfer the supernatant to new tube (stored for testing).
13. Wash the bead-Ab-Ag complex with 132 ul of washing solution 3 times. Separate with magnet every time.

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14. After placing in the magnet, remove the solution and add 66 ul of washing buffer, pipette gently.
15. Transfer the sample in new tube (avoid elution of proteins bound to tube).

    NB: *To store beads-Ab-Ag complex, place the solution in the magnet, remove the solution, add 13 ul of elution buffer (in kit) and 15 ul of 4x SDS sample buffer with fresh B ME. This mix should be freezed until usage.*

16. Remove the supernatant
17. Add 13 ul of elution buffer (in kit) and 15 ul of 4x SDS sample buffer with fresh 5% Beta-mercaptoethanol
18. Gently pipette the mixture
19. Heat at 100 C for 15 minute, let to cool

    NB: *If you will be performing a Western blot using rabbit antibodies (primary or secondary), do not heat the samples. Incubate at RT for 10 minutes with mixing.*

20. Place the tube in the magnet, collect the supernatant for subsequent step.
    NB: Loading the sample on SDS-PAGE should yield 3 bands (targeted protein, Ab heavy chain close to 50 KDa, and Ab light chain close to 25 KDa)

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