Product Information:

Mini-Protein Purification Kit (Cat.: PK-001)

Storage: 2-8°C

Kit contains (10 assays):
Bead Slurry (10ml), Dilution Buffer (250ml), Blocking Buffer (30ml), Elution Buffer (5ml), Neutralization Buffer (500ul).

Description:
This kit is designed for rapidly purifying the native functional antibodies, antigens or other biomolecules from cell/tissue lysates, hybridomas supernatants, sera, or other protein-protein interaction sources. This kit efficiently immobilizes antibodies, protein A/G, ligands to the agarose-based bead slurry without using the hazardous chemicals (e.g., sodium cyanoborohydride, cyanogen bromide), allows specific affinity of interesting proteins more than 25mg/mL (slurry).The immobilized antibody/ligand-bead slurry is compatible to column and batch affinity chromatography techniques and can be used/reused for multiple affinity purification procedures (e.g., monoclonal antibody purification, recombinant protein purification).

Procedure:

Additional Materials required prior to procedure:

- ✓ Empty columns (e.g., 2 mL) and compatible centrifuge/collection tubes (e.g. 15ml tubes)
- ✓ Antibodies or ligands solution: Dilute 0.5-10 mg (100ul-500ul) antibodies in 2-3 ml of dilution buffer.
- ✓ Sample preparation: Dilute sample 1:1 in dilution buffer.

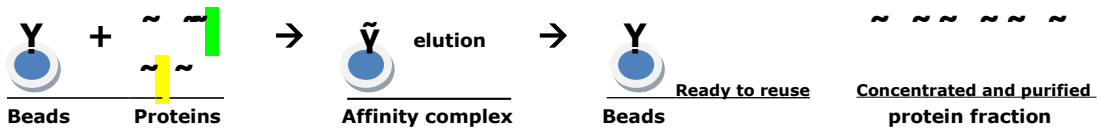
Immobilization procedure:

1. Invert the bottle of bead slurry several times and pipette 1-2ml bead slurry into a 2 ml spin column with bottom cap.
2. Place the column upright and allow the resin to settle at room temperature for 2-5 minutes.
3. Remove the bottom cap and place column in a collection tube. Centrifuge column at 1,000 × *g* for 1 minute and discard the flow-through. **Note:** Do not allow resin bed to become dry at any time during the procedure.
4. Add 2 ml of dilution buffer to column, centrifuge at 1,000 × *g* for 1 minute and discard the flow-through. Repeat once.
5. Replace the bottom cap and add 2 ml of the antibody/ligand solution to the column.
6. Replace the top cap and incubate for 45-60 minutes at room temperature with gentle shaking.
7. Remove the bottom/ top caps and place column in a collection tube. Centrifuge column at 1,000 × *g* for 1 minute and discard the flow-through.
8. Add 2 ml of dilution buffer to the column to wash out all the non-bound antibodies/ligands. Place the column in a new collection tube and centrifuge at 1,000 × *g* for 1 minute. Discard the flow-through. Repeat this step once.
9. Replace the bottom cap and add 3 ml of blocking buffer into the column. Replace the top cap.
10. Incubate for 15 minutes at room temperature with gentle shaking.
11. Remove bottom/top caps and place the column in a new collection tube, centrifuge at 1,000 × *g* for 1 minute. Discard the flow-through.
12. Wash column with 2 ml dilution buffer for 3 times. Centrifuge at 1,000 × *g* for 1 minute and discard the flow-through.

Affinity procedure:

13. Replace the bottom cap on the column. Add 2-4mL sample into column and replace the top cap.
14. Incubate the column for 1-2 hours at room temperature or overnight at 4ºC with gentle shaking.
15. Remove top/bottom caps and place column in new collection tube. Centrifuge the column at 1,000 × *g* for 1 minute. Discard the flow-through.
16. Wash column with 2 ml dilution buffer for 3 times. Centrifuge at 1,000 × *g* for 1 minute and discard the flow-through.
17. Add 250-500ul of Elution Buffer into column and place column in new collection tube containing 25-50ul of Neutralization Buffer. Centrifuge at 1,000 × *g* for 1 minute and save the **flow-through** (protein fractions). Repeat this step for five times and collect all the flow-through for determining which fraction is the best.
18. Measure the protein concentration at 280 nm or by Bradford assay.

Note 1: Regenerate the column immediately after elution by washing column with 3 ml of dilution buffer for twice to remove any residual protein fraction and neutralize the elution buffer on the column. Add 3 ml of dilution buffer to the column and cap the top/ bottom. Store the column upright at 4°C. For another affinity procedure, continue the step 12-18.
Note 2: For month storage, add 0.05% sodium azide or other preservative into column and store column upright at 4°C.



Precautions and Disclaimer:
This product and procedure described are intended for R&D use only. Purchase of this product does not convey a license to perform any patented process.

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