



Sino Biological  
Biological Solution Specialist

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# **Anti-CYB561D1 Magnetic Beads Immunoprecipitation (IP) Kit**

**Catalog Number: MB203730-T36**

Please read this instruction manual carefully before using the product

## Product Contents

|   | Contents                                    | Package 1 (20 Tests)                         | Package 2 (100 Tests) | Storage             |
|---|---------------------------------------------|----------------------------------------------|-----------------------|---------------------|
| 1 | Anti-CYB561D1 Magnetic Beads <sup>1,3</sup> | 1 mL                                         | 5 mL                  | 2-8°C for 12 months |
| 2 | NP40 Cell Lysis Buffer <sup>2</sup>         | 4 mL                                         | 22 mL                 | -20°C for 12 months |
| 3 | 5×TBST (pH7.4)                              | Required but not supplied                    |                       |                     |
| 4 | 1×TBST (pH7.4)                              | Required but not supplied                    |                       |                     |
| 5 | ddH <sub>2</sub> O                          | Required but not supplied                    |                       |                     |
| 6 | Alkaline Elution Buffer                     | 3 mL                                         | 15 mL                 | 2-8°C for 12 months |
| 7 | Acidity Elution Buffer                      | 3 mL                                         | 15 mL                 | 2-8°C for 12 months |
| 8 | Neutralization Buffer                       | 2 mL                                         | 8 mL                  | 2-8°C for 12 months |
| 9 | Magnetic Separator                          | One Simple Magnetic Separator (Cat# MAGS001) |                       |                     |

[1] The IP KIT contains anti-CYB561D1 Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).

**[2] Using NP-40 cell lysis buffer in the kit is required, otherwise, the magnetic beads may be precipitated.**

[3] Immunomagnetic Beads kits are shipped at ambient temperature in which immunomagnetic beads are provided in liquid buffer.

## Product Description

The Anti-CYB561D1 Immunomagnetic Beads, conjugated with Anti-CYB561D1 antibody, are used for immunoprecipitation (IP) of CYB561D1 proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing CYB561D1 proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound CYB561D1 proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

## Antibody Information

|                           |                                                                                                                              |
|---------------------------|------------------------------------------------------------------------------------------------------------------------------|
| <b>Antibody:</b>          | CYB561D1 Antibody, Rabbit PAb, Antigen Affinity Purified (Cat# 203730-T36)                                                   |
| <b>Immunogen:</b>         | E. coli-derived Human CYB561D1 fragment                                                                                      |
| <b>Isotype:</b>           | Rabbit IgG                                                                                                                   |
| <b>Specificity:</b>       | Human CYB561D1                                                                                                               |
| <b>Preparation:</b>       | Produced in rabbits immunized with E. coli-derived Human CYB561D1 fragment, and purified by antigen affinity chromatography. |
| <b>Applications:</b>      | IP, Minimum Protein Purification                                                                                             |
| <b>Alternative Names:</b> | CYB561D1                                                                                                                     |

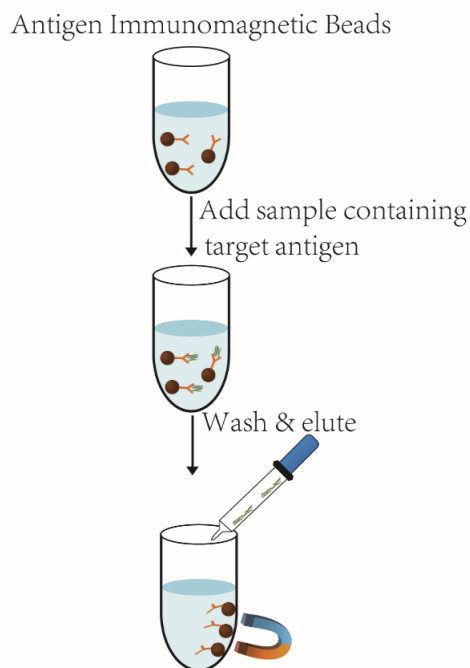


Fig. 1 Immunoprecipitation (IP) Protocol

## Protocol

The protocol (Fig. 1) uses 50  $\mu$ L Anti-CYB561D1Immunomagnetic Beads, but this can be scaled up or down as required.

### Cell Lysis

Cells may be lysed using any standard cell lysis protocol in accordance with your starting materials. **We suggest using NP40 Cell Lysis Buffer (supplied with kit).**

### Immunoprecipitate Target Antigen

1. Add 50  $\mu$ L of Immunomagnetic Beads into a 1.5 mL microcentrifuge tube.
2. Add 150  $\mu$ L of 1 $\times$  TBST buffer to the Immunomagnetic Beads and gently vortex to mix.
3. Place the tube into a Magnetic Separator to collect the beads against the wall side of the tube. Remove and discard the supernatant.
4. Add 1 mL of 1 $\times$  TBST buffer to the tube. Invert the tube several times or gently vortex to mix for 1 min. Collect Immunomagnetic Beads with a Magnetic Separator. Remove and discard the supernatant.
5. Add the sample containing target protein (Cell lysate: 0.5-1mg; Recombinant protein: 5-25  $\mu$ g) to the pre-washed Immunomagnetic Beads, add 1 $\times$  TBST buffer until final volume to 200-500  $\mu$ L, and incubate at 37 $^{\circ}$ C for 20-30 min (or at room temperature for 2-3h) with mixing.
6. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbounded sample and save for analysis.
7. Add 300  $\mu$ L of 5 $\times$  TBST buffer to the tube and gently mix. Collect the Immunomagnetic Beads and discard the supernatant. Repeat this wash twice.
8. Add 300  $\mu$ L of ddH<sub>2</sub>O to the tube and gently mix. Collect the Immunomagnetic Beads on a Magnetic Separator and discard the supernatant.

### Elute Target Antigen.

#### A. Alkaline Elution

1. Add 100  $\mu$ L of Alkaline Elution buffer to the tube.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the sample, add 50  $\mu$ L of Neutralization Buffer for each 100  $\mu$ L of eluate.

#### B. Acidity Elution

1. Add 100  $\mu$ L Acidity Elution Buffer.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 15  $\mu$ L of Neutralization Buffer for each 100  $\mu$ L of eluate.

#### C. Denaturing Elution

1. Add 10  $\mu$ L of 2 $\times$  SDS-PAGE Sample Loading Buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100  $^{\circ}$ C for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the antigen.

### General Test System of Sino Biological Inc. (for reference) :

|                | Recombinant Protein                                                  | Cell Lysate |
|----------------|----------------------------------------------------------------------|-------------|
| Sample Quality | 10 $\mu$ g add into 0.5mg cell lysate (without interfering proteins) | 0.5mg       |
| Final Volume   | 300 $\mu$ L                                                          |             |
| Incubate Time  | Room temperature, 2h                                                 |             |
| Elute          | Using 10 $\mu$ L of 2 $\times$ SDS-PAGE Sample Loading Buffer        |             |

## Reference Information

### Related Products

| Products                                                                         | Cat No.  |
|----------------------------------------------------------------------------------|----------|
| Magnetic Separator-1.5 (2 tubes)                                                 | MAGS001  |
| Immunoprecipitation Kit<br>-Immunomagnetic Beads Protein A Kit                   | BA10600  |
| Immunoprecipitation Kit<br>-Immunomagnetic Beads Protein G Kit                   | BG13103  |
| Immunoprecipitation Kit<br>-Immunomagnetic Beads Protein L Kit                   | BL11044  |
| Immunoprecipitation Kit<br>-Immunomagnetic Beads Protein A/G Kit                 | BAG001   |
| Immunoprecipitation Kit<br>-Anti-DYKDDDDK(Flag®) Tag<br>Immunomagnetic Beads Kit | TB101274 |
| Immunoprecipitation Kit<br>-Anti-GFP Tag Immunomagnetic Beads Kit                | TB13105  |
| Immunoprecipitation Kit<br>-Anti-Myc Tag Immunomagnetic Beads Kit                | TB100029 |
| Immunoprecipitation Kit<br>-Anti-HA Tag Immunomagnetic Beads Kit                 | TB100028 |
| Immunoprecipitation Kit<br>-Anti-V5 Tag Immunomagnetic Beads Kit                 | TB100378 |
| Immunoprecipitation Kit<br>-Anti-GST Tag Immunomagnetic Beads Kit                | TB11213  |
| Magpains™ His-Tag Immunoprecipitation Kit                                        | TBN001   |

### Trouble Shooting

| Problem                          | Possible Cause                      | Solution                                                        |
|----------------------------------|-------------------------------------|-----------------------------------------------------------------|
| Little or no protein is detected | Protein degraded                    | Include protease inhibitors (PMSF) in the lysis buffer          |
|                                  |                                     | Use new lysate or lysate stored at -80° C                       |
|                                  | No or minimal protein was expressed | Verify protein expression by SDS-PAGE or Western blot           |
|                                  |                                     | Analysis of the lysate using an positive control as a reference |

| Problem                              | Possible Cause                                                           | Solution                                                                                       |
|--------------------------------------|--------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Little or no protein is detected     | No or minimal protein was expressed                                      | Increase the amount of lysate used for IP/Co-IP                                                |
|                                      |                                                                          | Use a more sensitive detection system                                                          |
| Magnetic Beads aggregated            | Magnetic Beads were frozen or centrifuged                                | Handle the Beads as directed in the instructions                                               |
|                                      | Buffer was incompatible with magnetic beads                              |                                                                                                |
|                                      | Detergent was not added to the wash and bind solutions                   |                                                                                                |
| Failure to co-IP interacting protein | Wash conditions were too stringent for the weak or transient interaction | Reduce the number of washes                                                                    |
|                                      |                                                                          | Lower the ionic strength of the wash buffer                                                    |
|                                      | Interacting protein was expressed at a low level                         | Apply additional protein sample                                                                |
|                                      |                                                                          | Use a more sensitive detection system                                                          |
|                                      | Buffer system was not optimal for the protein: protein interaction       | Optimize the co-IP buffer                                                                      |
|                                      | Insufficient sample was loaded on the gel for Western blot detection     | Elute sample in 30% acetonitrile 0.5% formic acid, then                                        |
|                                      |                                                                          | Bring the sample back up in SDS-PAGE Sample Loading Buffer and load entire elution fraction on |