



**Sino Biological Inc.**  
Biological Solution Specialist

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# **Immunoprecipitation/IP Kit- Anti-NOX4 Immunomagnetic Beads**

**Catalog Number: MB102385-T04**

Please read this instruction manual carefully before using the product

Product Contents

| Contents                                      | Package 1                                    | Package 2                                  | Storage            |
|-----------------------------------------------|----------------------------------------------|--------------------------------------------|--------------------|
| Anti-NOX4 Immunomagnetic Beads <sup>1 3</sup> | 1 mL                                         | 5 mL                                       | 2-8℃ for 12 months |
| NP40 Cell Lysis Buffer                        | 4 mL                                         | 22 mL                                      | -20℃ for 12 months |
| 5×TBST (pH7.4)                                | Required but not supplied                    |                                            |                    |
| 1×TBST (pH7.4)                                | Required but not supplied                    |                                            |                    |
| ddH <sub>2</sub> O                            | Required but not supplied                    |                                            |                    |
| Alkaline Elution Buffer                       | 3 mL                                         | 15 mL                                      | 2-8℃ for 12 months |
| Acidity Elution Buffer                        | 3 mL                                         | 15 mL                                      | 2-8℃ for 12 months |
| Neutralization Buffer                         | 2 mL                                         | 8 mL                                       | 2-8℃ for 12 months |
| Magnetic Separator                            | Not included (refer related product MAGS001) | One MAGS001 included in China <sup>2</sup> |                    |

- [1] The IP KIT contains anti-NOX4 Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).
- [2] The Magnetic Separator cannot be included for oversea customers due to shipment prohibition.
- [3] Immunomagnetic Beads kits are shipped at ambient temperature in which immunomagnetic beads are provided in liquid buffer.

Product Description

The Anti-NOX4 Immunomagnetic Beads, conjugated with Anti-NOX4 antibody, are used for immunoprecipitation (IP) of NOX4 proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing NOX4 proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound NOX4 proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

Antibody Information

**Antibody:** NOX4 Antibody, Rabbit PAb, Antigen Affinity Purified(102385-T04)

**Immunogen:** A synthetic peptide corresponding to the center region of the Human NOX4

**Clone ID:**

**Isotype:** Rabbit IgG

**Specificity:** Human NOX4

**Guaranteed Applications:** IP, Minimum Protein Purification

**Preparation:** Produced in rabbits immunized with a synthetic peptide corresponding to the center region of the Human NOX4, and purified by antigen affinity chromatography.



Fig. 1 Immunoprecipitation (IP) Protocol

## Protocol

The protocol (Fig. 1) uses 50  $\mu\text{L}$  Anti-NOX4Immunomagnetic 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  $\mu\text{L}$  of Immunomagnetic Beads into a 1.5 mL microcentrifuge tube.
2. Add 150  $\mu\text{L}$  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 ( $\sim 100\text{ }\mu\text{g}$  of protein in 100  $\mu\text{L}$ ) to the pre-washed Immunomagnetic Beads, add 400  $\mu\text{L}$  of  $1\times$  TBST buffer and incubate at room temperature for 30 min with mixing.
6. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbounded sample and save for analysis.
7. Add 300  $\mu\text{L}$  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  $\mu\text{L}$  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 Protocols

1. Add 100  $\mu\text{L}$  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  $\mu\text{L}$  of Neutralization Buffer for each 100  $\mu\text{L}$  of eluate.

#### B. Acidity Elution

1. Add 100  $\mu\text{L}$  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  $\mu\text{L}$  of Neutralization Buffer for each 100  $\mu\text{L}$  of eluate.

#### C. Elution Using Sample Buffer

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

## 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>-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 |

### 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 |
|                                  |                                     | Increase the amount of lysate used for IP/Co-IP                 |
|                                  |                                     | Use a more sensitive detection system                           |
|                                  |                                     |                                                                 |
|                                  |                                     |                                                                 |

| Problem                              | Possible Cause                                                           | Solution                                                                               |
|--------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------------|
| 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 Buffer and load entire elution fraction on |