# MagIso™ Anti-SARS-CoV-2 (2019-nCoV) Spike S2 Magnetic Particles Immunoprecipitation (IP) Kit

**Catalog Number: WHK-SN026**

Please read this instruction manual carefully before using the product

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## Product Contents

| | Contents | Package 1 (20 Tests) | Package 2 (100 Tests) | Storage |
|---|---|---|---|---|
| 1 | Anti-SARS-CoV-2 (2019-nCoV) Spike S2 Magnetic Beads[1 3] | 1 mL | 5 mL | 2-8°C for 12 months |
| 2 | NP40 Cell Lysis Buffer[2] | 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 | $ddH_2O$ | 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 |

[1] The IP KIT contains anti-SARS-CoV-2 (2019-nCoV) Spike S2 Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).

**[2] Using NP-40 cell lysate 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-SARS-CoV-2 (2019-nCoV) Spike S2 Immunomagnetic Beads, conjugated with Anti-SARS-CoV-2 (2019-nCoV) Spike S2 antibody, are used for immuneprecipitation (IP) of SARS-CoV-2 (2019-nCoV) Spike S2 proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing SARS-CoV-2 (2019-nCoV) Spike S2 proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound SARS-CoV-2 (2019-nCoV) Spike S2 proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

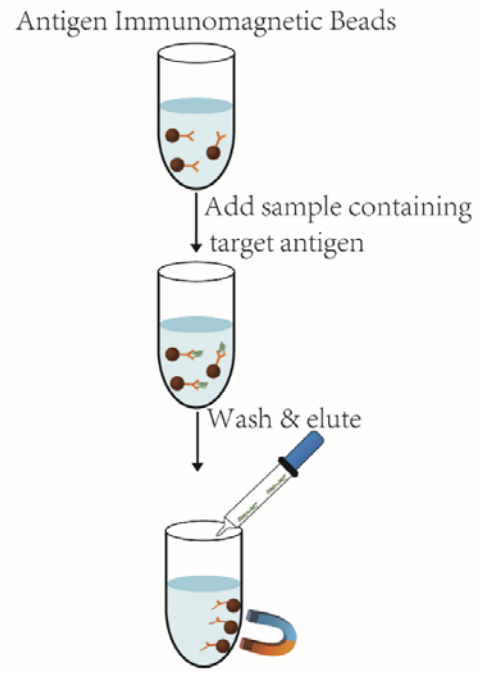


Fig. 1 Immunoprecipitation (IP) Protocol

## Antibody Information

**Antibody:** SARS-CoV-2 (2019-nCoV) Spike S2 Antibody, Rabbit PAb, Antigen Affinity Purified

**Immunogen:** Recombinant SARS-CoV-2 /2019-nCoV Spike/S2 Protein

**Isotype:** Rabbit IgG

**Specificity:** SARS-CoV-2 (2019-nCoV) Spike S2 (ECD, His tag)

**Preparation:** Produced in rabbits immunized with recombinant SARS-CoV-2 / 2019-nCoV Spike/S2 Protein. The specific IgG was purified by SARS-CoV-2 / 2019-nCoV Spike/S2 affinity chromatography.

**Applications:** IP, Minimum Protein Purification

## Protocol

The protocol (Fig. 1) uses 50 µL Anti-SARS-CoV-2 (2019-nCoV) Spike S2Immunomagnetic 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 µL of Immunomagnetic Beads into a 1.5 m microcentrifuge tube.
2. Add 150 µL of 1×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×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 µg) to the pre-washed Immunomagnetic Beads, add 1×TBST buffer until final volume to 200-500 µL, and incubate at 37°C for 20-30 min (or at room temperature for 2-3h) with mixing.
5. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbounded sample and save for analysis.
6. Add 300 µL of 5×TBST buffer to the tube and gently mix. Collect the Immunomagnetic Beads and discard the supernatant. Repeat this wash twice.
7. Add 300 µL of dd$H_2O$ 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 µL 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 µL of Neutralization Buffer for each 100 µL of eluate.

*B. Acidity Elution*

1. Add 100 µL 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 µL of Neutralization Buffer for each 100 µL of eluate.

*C. Denaturing Elution*

1. Add 10 µL of 2×SDS-PAGE Sample Loading 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.

**General Test System (for reference) :**

| | **Recombinant Protein** | **Cell Lysate** |
|---|---|---|
| Sample Quality | 10µg add into 0.5mg cell lysate (without interfering proteins) | 0.5mg |
| Final Volume | 300µL | |
| Incubate Time | Room temperature, 2h | |
| Elute | Using 10 µL of 2×SDS-PAGE Sample Loading Buffer | |

**Trouble Shooting**

| 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 Loading Buffer and load entire elution fraction on |

| 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<br>Use a more sensitive detection system |