



Sino Biological  
Biological Solution Specialist

---

# **Anti-HIV-1 gp140 (group M, subtype CRF07\_BC) Magnetic Beads Immunoprecipitation (IP) Kit**

**Catalog Number: MB11677-MM05T**

Please read this instruction manual carefully before using the product

## Product Contents

|   | Contents                                                                   | Package 1 (20 Tests)                         | Package 2 (100 Tests) | Storage             |
|---|----------------------------------------------------------------------------|----------------------------------------------|-----------------------|---------------------|
| 1 | Anti-HIV-1 gp140 (group M, subtype CRF07_BC) 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-HIV-1 gp140 (group M, subtype CRF07\_BC) 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-HIV-1 gp140 (group M, subtype CRF07\_BC) Immunomagnetic Beads, conjugated with Anti-HIV-1 gp140 (group M, subtype CRF07\_BC) antibody, are used for immunoprecipitation (IP) of HIV-1 gp140 (group M, subtype CRF07\_BC) proteins which expressed in vitro expression systems and bacterial and mammalian cell lysates.

For IP, the beads are added to a sample containing HIV-1 gp140 (group M, subtype CRF07\_BC) proteins to form a bead-protein complex. The complex is removed from the solution manually using a Magnetic Separator. The bound HIV-1 gp140 (group M, subtype CRF07\_BC) proteins are dissociated from the Immunomagnetic Beads using an elution buffer.

Antigen Immunomagnetic Beads

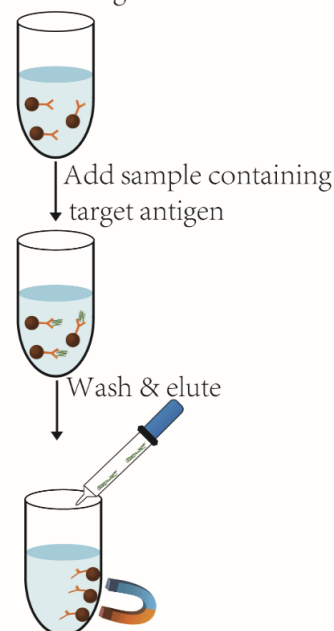


Fig. 1 Immunoprecipitation (IP) Protocol

## Antibody Information

|                           |                                                                                                                                                                                                                                                                                                                                                  |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Antibody:</b>          | HIV-1 gp140 (group M, subtype CRF07_BC)<br>Antibody, Mouse MAb (Cat# 11677-MM05T)                                                                                                                                                                                                                                                                |
| <b>Immunogen:</b>         | Recombinant HIV-1 gp140 (group M, subtype CRF07_BC) Protein (Catalog#11677-V08H)                                                                                                                                                                                                                                                                 |
| <b>Clone ID:</b>          | 05                                                                                                                                                                                                                                                                                                                                               |
| <b>Isotype:</b>           | Mouse IgG1                                                                                                                                                                                                                                                                                                                                       |
| <b>Specificity:</b>       | HIV-1 gp140 (group M, subtype CRF07_BC)                                                                                                                                                                                                                                                                                                          |
| <b>Preparation:</b>       | This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, recombinant HIV-1 gp140 (group M, subtype CRF07_BC) (Catalog#11677-V08H; Val30-Tyr677). The IgG fraction of the cell culture supernatant was purified by Protein A affinity chromatography. |
| <b>Applications:</b>      | IP, Minimum Protein Purification                                                                                                                                                                                                                                                                                                                 |
|                           | <i>Notes: The applications have not been validated with corresponding viruses.</i>                                                                                                                                                                                                                                                               |
| <b>Alternative Names:</b> | gp140                                                                                                                                                                                                                                                                                                                                            |

## Protocol

The protocol (Fig. 1) uses 50 µL Anti-HIV-1 gp140 (group M, subtype CRF07\_BC) Immunomagnetic 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 mL 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.
6. Collect the Immunomagnetic Beads with a Magnetic Separator, remove the unbounded sample and save for analysis.
7. 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.
8. Add 300 µL 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 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 of Sino Biological Inc. (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                |             |

## 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  |
| Magpoins™ 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 |