



**Sino Biological Inc.**  
Biological Solution Specialist

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# Immunoprecipitation/IP Kit- Anti-VEGF / VEGFA Immunomagnetic Beads

**Catalog Number: MB100253-T36**

Please read this instruction manual carefully before using the product

## Product Contents

Contents	Package 1	Package 2	Storage
Anti-VEGF / VEGFA Immunomagnetic Beads <sup>1,3</sup>	1 mL	5 mL	2-8°C for 12 months
NP40 Cell Lysis Buffer	4 mL	22 mL	-20°C 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°C for 12 months
Acidity Elution Buffer	3 mL	15 mL	2-8°C for 12 months
Neutralization Buffer	2 mL	8 mL	2-8°C 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-VEGF / VEGFA Immunomagnetic Beads(2 mg/mL) in phosphate buffered saline (PBS, pH 7.4) with sodium azide (0.1%).

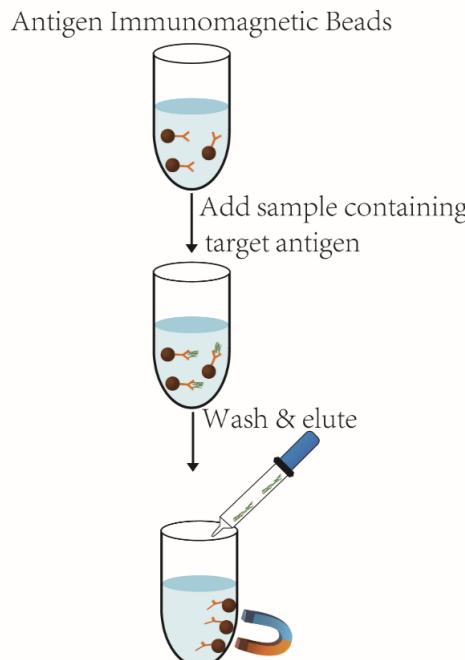
[2] The Magnetic Separator cannot be included for oversea customers due to shipment prohibition.

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

## Product Description

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

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



## Antibody Information

**Antibody:** VEGFA Antibody, Rabbit PAb, Antigen Affinity Purified(100253-T36)

**Immunogen:** A synthetic peptide corresponding to the center region of the human VEGFA

**Clone ID:**

**Isotype:** Rabbit IgG

**Specificity:** human VEGFA

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

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

Fig. 1 Immunoprecipitation (IP) Protocol

## Protocol

The protocol (Fig. 1) uses 50  $\mu$ L Anti-VEGF / VEGFAImmunomagnetic 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 (~100  $\mu$ g of protein in 100  $\mu$ L) to the pre-washed Immunomagnetic Beads, add 400  $\mu$ 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  $\mu$ 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 Protocols

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. Elution Using Sample Buffer

1. Add 100  $\mu$ 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 -Immunomagnetic Beads Protein A Kit	BA10600
Immunoprecipitation Kit -Immunomagnetic Beads Protein G Kit	BG13103
Immunoprecipitation Kit -Immunomagnetic Beads Protein L Kit	BL11044
Immunoprecipitation Kit -Anti-DYKDDDDK(Flag®) Tag Immunomagnetic Beads Kit	TB101274
Immunoprecipitation Kit -Anti-GFP Tag Immunomagnetic Beads Kit	TB13105
Immunoprecipitation Kit -Anti-Myc Tag Immunomagnetic Beads Kit	TB100029
Immunoprecipitation Kit -Anti-HA Tag Immunomagnetic Beads Kit	TB100028
Immunoprecipitation Kit -Anti-V5 Tag Immunomagnetic Beads Kit	TB100378

Problem	Possible Cause	Solution
Magnetic Beads aggregated	Magnetic Beads were frozen or centrifuged Buffer was incompatible with magnetic beads Detergent was not added to the wash and bind solutions	Handle the Beads as directed in the instructions
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	
Failure to co-IP interacting protein	Buffer system was not optimal for the protein: protein interaction	Optimize the co-IP buffer
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 a positive control as a reference Increase the amount of lysate used for IP/Co-IP Use a more sensitive detection system
		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

### 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 a positive control as a reference Increase the amount of lysate used for IP/Co-IP Use a more sensitive detection system