

# Anti-GST Tag Magnetic Beads Immunoprecipitation (IP) Kit



Sino Biological  
Biological Solution Specialist

Catalog Number: TB11213

## Product Contents

Contents	TB11213-20	TB11213-100	Storage
GST Tag Immunomagnetic Beads <sup>1</sup>	1 mL	5 mL	2-8°C for 12 months
NP40 Cell Lysis Buffer <sup>2</sup>	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		
GST Tag Positive Cell Lysis	300 µg	300 µg	-20°C for 12 months
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

[1] GST Tag Immunomagnetic Beads contain 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.

## Product Description

The GST Tag Immunomagnetic Beads, conjugated with GST Antibody, are used for Immunoprecipitation / IP of GST-tagged proteins which expressed in vitro expression systems. For IP, the Immunomagnetic Beads are added to a sample containing GST-tagged proteins to form an Immunomagnetic Beads-protein complex. The complex is removed from the solution manually against a Magnetic Separator. The bound GST-tagged proteins are dissociated from the Immunomagnetic Beads using an Elution Buffer.

## Antibody Information

**Antibody:** GST Tag Antibody, Mouse MAb ([11213-MM01](#))

**Immunogen:** Recombinant GST protein.

**Clone ID:** MM01

**Isotype:** IgG1

**Specificity:** Recognize GST Tag in fusion proteins.

**Preparation:** 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 Glutathione S-transferase (GST). The IgG fraction of the cell culture supernatant was purified by Protein A affinity chromatography.

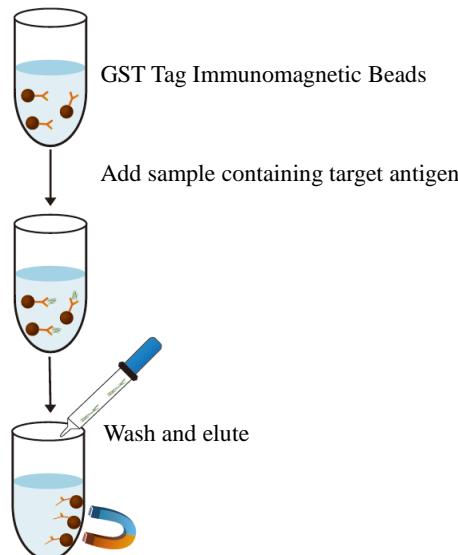


Fig. 1 Immunoprecipitation (IP) Protocol

## Protocol

The protocol (Fig. 1) uses 50  $\mu$ L GST Tag 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).** Add protease inhibitor (such as PMSF at 1mM) if needed.

### 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 Immunomagnetic Beads against the side wall 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 the 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 unbound 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 Beads on a Magnetic Separator and discard the supernatant.

### Elute Target Antigen

#### A. Alkaline Elution Protocol

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 Protocol

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.

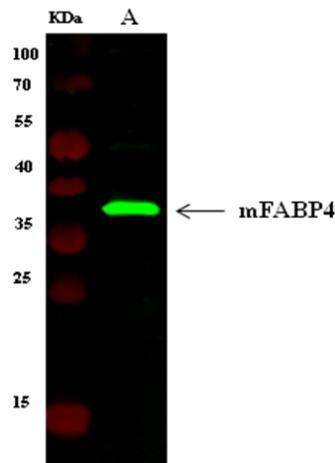
4. To neutralize the low pH, add 50  $\mu$ L of Neutralization Buffer for each 100  $\mu$ L of eluate.

### 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 $^{\circ}$ C for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the antigen.

### Usage of positive cell lysate

The positive cell lysis can be used in Western Blotting or Immunoprecipitation.



Items	Lane
Sample (30 $\mu$ g) (Whole cell lysate)	A
	GST-mFABP4-Flag
Beads	SBI Anti-GST Tag Immunomagnetic Beads-30 $\mu$ L
WB detection antibody	Anti-GST Tag Antibody, Mouse MAb (11213-MM01) at 10 $\mu$ g/mL
Gel	13% SDS-PAGE reducing gel
Secondary antibody	Dylight 800-labeled antibody to mouse IgG (H+L), at 1:15000 dilution
Observed band size	38 kDa

## Protocol-中文版

如图1所示, 取GST tag 免疫磁珠50μL (实际取用量可根据样品中目的蛋白的含量适当增减)

### Cell Lysis(细胞裂解液制备)

细胞裂解液制备可参照标准细胞裂解液制备步骤操作。我们**建议**采用**本试剂盒提供的NP40细胞裂解试剂** (如有需要可加入蛋白酶抑制剂PMSF至1mM)

### Immunoprecipitate Target Antigen (免疫沉淀步骤)

1. 取 50 μL GST tag 免疫磁珠放入 1.5 mL 离心管。
2. 向上述离心管中加入 150 μL 1× TBST 轻轻颠倒混匀 1 分钟。
3. 将混匀后的离心管放入磁力架, 待吸附完成弃去上清, 重复 2 次。
4. 向上述离心管中加入制备好的细胞裂解液 (蛋白含量约为 1mg/mL) 然后再加入 400 μL 1×TBST 在旋转混合仪中 37°C 孵育 30 分钟。
5. 孵育完成后将离心管放入磁力架收集磁珠, 上清可丢弃亦可收集用于后续分析 (如有必要)。
6. 向装有磁珠的离心管中加入 300 μL 5× TBST 轻轻颠倒后弃上清, 洗磁珠 3 次。
7. 向装有磁珠的离心管中加入 300 μL ddH<sub>2</sub>O 轻轻颠倒混匀弃上清 (根据实验目的可适当增加清洗次数)。

### Elute Target Antigen (抗原洗脱)

#### A. Alkaline Elution Protocol (碱性洗脱)

1. 向装有磁珠的离心管加入 100 μL Alkaline Elution Buffer。
2. 在旋转混合仪上室温轻轻颠倒孵育 5 -10 分钟。
3. 将装有磁珠的离心管放入磁力架收集上清 (注: 含有抗原勿丢弃)。
4. 如需中和碱性洗脱液碱性可向 100 μL 碱性洗脱液中加入 Neutralization Buffer (中和液) 50 μL 或调整 PH 至中性。

#### B. Acidity Elution Protocol (酸性洗脱)

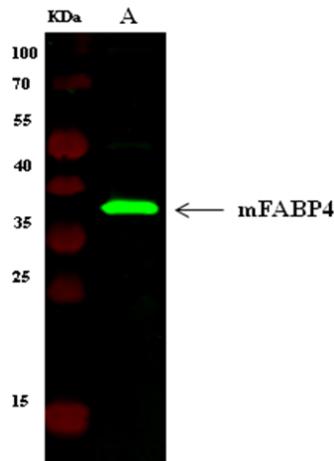
1. 向装有磁珠的离心管中加入 100 μL Acidity Elution Buffer.
2. 在旋转混合仪上室温轻轻颠倒孵育 5 -10 分钟。
3. 将装有磁珠的离心管放入磁力架收集上清 (注意: 含有抗原勿丢弃)。
4. 如需中和酸性洗脱液酸性可向 100 μL 碱性洗脱液中加入 Neutralization Buffer (中和液) 50 μL 或调整 PH 至中性。

#### C. Elution Using Sample Buffer (Loading buffer 洗脱)

1. 如进行 western blotting 检测可加入 100 μL SDS-PAGE loading Buffer, 混匀后 95-100°C 煮 5-10 分钟。
2. 煮后将离心管放入磁力架, 收集上清 (注意: 有抗原勿丢弃) 用于 SDS-PAGE 电泳。

### Usage of positive cell lysate (阳性细胞裂解液使用)

本试剂盒提供的阳性细胞裂解液, 既可用做 Western Blotting 阳性样品, 亦可作为免疫沉淀阳性样品。



Items	Lane
Sample (30 μg) (Whole cell lysate)	A GST-mFABP4-Flag
Beads	SBI Anti-GST Tag Immunomagnetic Beads-30 μL
WB detection antibody	Anti-GST Tag Antibody, Mouse MAb (11213-MM01) at 10 μg/mL
Gel	13% SDS-PAGE reducing gel
Secondary antibody	Dylight 800-labeled antibody to mouse IgG (H+L), at 1:15000 dilution
Observed band size	38 kDa

## Reference Information

### Related Products

Products	Cat No.
Magnetic separator-1.5 (2 tubes) for IP	MAGS001
Protein A Magnetic Beads	BA10600
Immunoprecipitation (IP) Kit	
Protein G Magnetic Beads	BG13103
Immunoprecipitation (IP) Kit	
Protein L Magnetic Beads	BL11044
Immunoprecipitation (IP) Kit	
ProteinA/ G Magnetic Beads	BAG001
Immunoprecipitation (IP) Kit	
Anti-MYC Tag Magnetic Beads	TB100029
Immunoprecipitation (IP) Kit	
Anti-GFP Tag Magnetic Beads	TB13105
Immunoprecipitation (IP) Kit	
Anti-HA Tag Magnetic Beads	TB100028
Immunoprecipitation (IP) Kit	
Anti-V5 Tag Magnetic Beads	TB100378
Immunoprecipitation (IP) Kit	
Anti-DYKDDDDK (Flag®) Tag Magnetic Beads	TB101274
Immunoprecipitation (IP) Kit	

### Trouble Shooting

Problem	Possible Cause	Solution
Little or no GST-tagged protein is detected	Tagged protein degraded	Include protease inhibitors (PMSF) in the lysis buffer
		Use new lysate or lysate stored at -80 °C
	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western blot
		analysis of the lysate using an GST-tagged 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 aggregated	Magnetic Beads were frozen or centrifuged
		Buffer was incompatible with magnetic beads
		Detergent was not added to the wash and bind solutions
	Wash conditions were too stringent for the weak or transient interaction	Handle the Beads as directed in the instructions
		Reduce the number of washes
	Interacting protein was expressed at a low level	Lower the ionic strength of the wash buffer
		Apply additional protein sample
		Use a more sensitive detection system
	Failure to co-IP interacting protein	Optimize the co-IP buffer
	Buffer system was not optimal for the protein: protein interaction	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

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