Anti-GFP Tag Magnetic Beads Immunoprecipitation (IP) Kit

Catalog Number: TB13105

Product Contents

Contents	TB13105-20	TB13105-100	Storage
GFP Tag Immunomagnetic Beads[1]	1 mL	5 mL	2-8℃ for 12 months
NP40 Cell Lysis Buffer[2]	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_2O	Required but not supplied		
GFP Tag Positive Cell Lysis	300 μg	300 μg	-20℃ for 12 months
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

[1] GFP 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 GFP Tag Immunomagnetic Beads, conjugated with GFP Antibody（13105-R208）, are used for Immuneprecipitation / IP of GFP-tagged proteins expressed in vitro expression systems. For IP, the Immunomagnetic Beads are added to a sample containing GFP-tagged proteins to form an Immunomagnetic Beads-protein complex. The complex is removed from the solution manually against a Magnetic Separator. The bound GFP-tagged proteins are dissociated from the Immunomagnetic Beads using an Elution Buffer.

Antibody Information

Antibody: GFP Antibody, Rabbit MAb (13105-R208)

Immunogen: Recombinant Aequorea victoria GFP protein (Catalog#13105-S07E)

Clone ID: R208

Isotype: IgG

Specificity: Aequorea victoria GFP.

Preparation: This antibody was obtained from a rabbit immunized with purified, recombinant Aequorea victoria GFP (Catalog#13105-S07E; AAB65663; Ser2-Lys238).

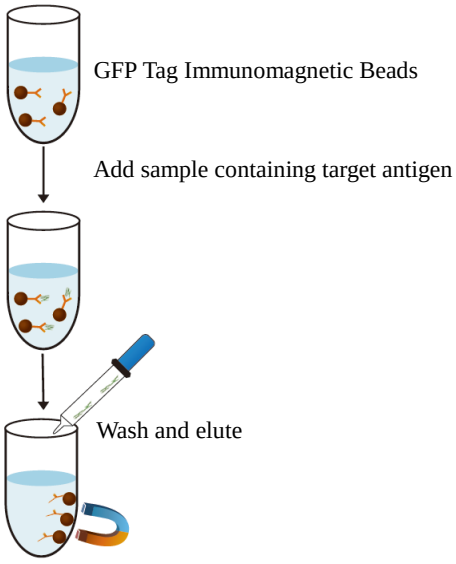


Fig. 1 Immunoprecipitation (IP) Protocol

Protocol

The protocol (Fig. 1) uses 50 µL GFP 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 prtease inhibitor (such as PMSF at 1mM) if needed.

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 Immunomagnetic Beads against the side wall 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 the Immunomagnetic Beads with a Magnetic Separator. Remove and discard the supernatant.
5. Add the sample containing target protein (~100 µg of protein in 100 µL) to the pre-washed Immunomagnetic Beads, add 400 µL of 1 ×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 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_2O to the tube and gently mix. Collect the Beads on a Magnetic Separator and discard the supernatant.

Elute Target Antigen

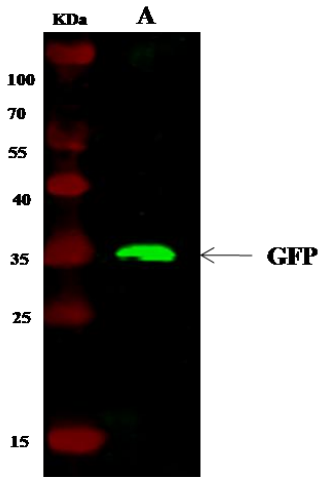
A. Alkaline Elution Protocol
1. Add 100 µL of Alkaline Elution Buffer to the tube.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the sample, add 50 µL of Neutralization Buffer for each 100 µL of eluate.

B. Acidity Elution Protocol
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 50 µL of Neutralization Buffer for each 100 µL of eluate.

C. Elution Using Sample Buffer
1. Add 100 µL of SDS-PAGE Sample Buffer to the tube.
2. Gently vortex to mix and incubate the sample at 95-100^{0}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 µg) (Whole cell lysate)	A
	GFP expressed by E.coli
Beads	SBI Anti-GFP tag Immunomagnetic Beads-30 µL
WB detection antibody	GFP tag Antibody, Rabbit MAb (13105-R208) 1 µg/mL
Gel	13% SDS-PAGE reducing gel
Secondary antibody	Dylight 800-labeled antibody to rabbit IgG (H+L), at 1:10000 dilution
Observed band size	35 kDa

Protocol-中文版

如图1所示，取GFP 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 GFP 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℃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_2O 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 100μ L 碱性洗脱液中加入 Neutralization Buffer（中和液）50μ L 或调整 PH 至中性。

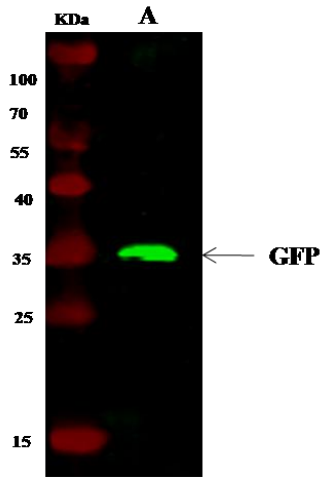
C. Elution Using Sample Buffer（Loading buffer 洗脱）

1. 如进行 western blotting 检测可加入 100 μL SDS-PAGE loading Buffer，混匀后 95-100^{0}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。



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Secondary antibody	Dylight 800-labeled antibody to rabbit IgG (H+L), at 1:10000 dilution
Observed band size	35 kDa

Reference Information

Related Products

Products	Cat No.
Magnetic separator-1.5 (2 tubes) for IP	MAGS001
Protein A Magnetic Beads Immunoprecipitation (IP) Kit	BA10600
Protein G Magnetic Beads Immunoprecipitation (IP) Kit	BG13103
Protein L Magnetic Beads Immunoprecipitation (IP) Kit	BL11044
ProteinA/ G Magnetic Beads Immunoprecipitation (IP) Kit	BAG001
Anti-MYC Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB100029
Anti-HA Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB100028
Anti-V5 Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB100378
Anti-GST Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB11213
Anti-DYKDDDDK (Flag®) Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB101274

Trouble Shooting

Problem	Possible Cause	Solution
Little or no GFP-tagged protein is detected	Tagged protein degraded	Include protease inhibitors (PMSF) in the lysis buffer
		Use new lysate or lysate stored at -80 ℃
	No or minimal tagged protein was expressed	Verify protein expression by SDS-PAGE or Western blot
		analysis of the lysate using an GFP-tagged positive control as a reference
		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 Buffer and load entire elution fraction on