Anti-HA Tag Magnetic Beads Immunoprecipitation (IP) Kit

Sino Biological
Biological Solution Specialist

Catalog Number: TB100028

Product Contents

Contents	TB100028-20	TB100028-100	Storage
HA 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		
HA 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
HA Synthetic Peptide	Not included (refer related product PP100028)		-20℃ for 12 months

[1] HA 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 HA Tag Immunomagnetic Beads, conjugated with HA tag Antibody (100028-MM15), are used for Immuneprecipitation / IP of HA-tagged proteins expressed in vitro expression systems. For IP, the Immunomagnetic Beads are added to a sample containing HA-tagged proteins to form a bead-protein complex. The complex is removed from the solution manually against a Magnetic Separator. The bound HA-tagged proteins are dissociated from the Immunomagnetic Beads using an Elution Buffer.

Antibody Information

Antibody: HA Tag Antibody, Mouse MAb (100028-MM15)

Immunogen: A synthetic peptide corresponding to the HA-tag sequence.

Clone ID: MM15

Specificity: Recognize N-terminal and C-terminal HA 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, a synthetic peptide corresponding to the HA-tag sequence. The IgG fraction of the cell culture supernatant was purified by Protein A affinity chromatography.

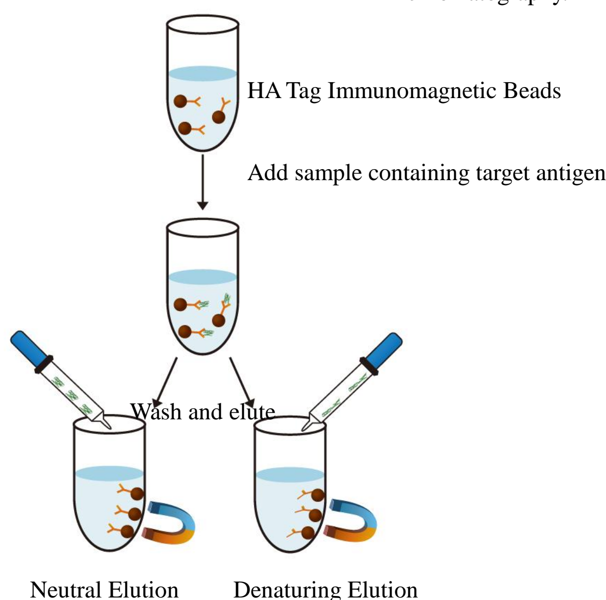


Fig. 1 Immunoprecipitation (IP) Protocol

Protocol

The protocol (Fig. 1) uses 50 µL HA 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. Neutral Elution Protocol

1. Prepare HA peptide (PP100028) at 1mg/mL in PBS.

1. Add 50 µL 1 mg/mL HA peptide to the Immunomagnetic Beads, gently vortex to mix and incubate the sample at 37°C on a rotator for 5-10 min. Elution may be performed at reduced temperatures, but lower yields may result.
2. Separate the Immunomagnetic Beads on a Magnetic Separator and save the supernatant containing the target antigen.
3. Repeat Elution step once for higher recovery.

B. 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.

C. Acidity Elution Protocol

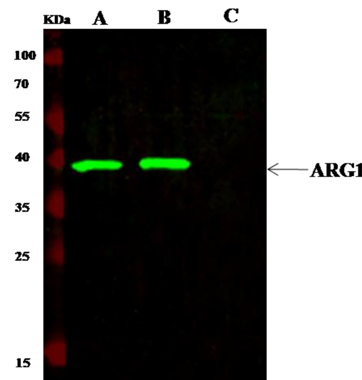
1. Add 100 µL Acidity Elution Buffer.
2. Gently vortex to mix and incubate the sample at room temperature on a rotator for 5-10 min.
3. Magnetically separate the Immunomagnetic Beads and save the supernatant containing the target antigen.
4. To neutralize the low pH, add 15 µL of Neutralization Buffer for each 100 µL of eluate.

D. 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	B	C
	HA-ARG1-myc Transfected 293	myc-ARG1-HA Transfected 293	pSTEP2 Transfection 293
Beads	SBI Anti-HA Tag Immunomagnetic Beads-30 µL		
WB detection antibody	Anti-HA Tag Antibody, Mouse MAb (100028-MM10) at 1 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	40 kDa		

Protocol-中文版

如图1所示，取HA 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 HA 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$_2$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. Neutral Elution Protocol（中性洗脱）

1. 用 PBS 配制 HA tag 多肽洗脱液 (PP101274) 为 1mg/mL

2. 向装有磁珠的离心管中加入 50 µL HA tag 多肽洗脱液， 在旋转混合仪上 37 ºC 孵育 5-10 分钟（低于 37 ºC 则需适当延长孵育时间）。

3. 将装有磁珠的离心管放入磁力架，收集上清（注：含有目的抗原勿丢弃）。

4. 如有必要可重复上述步骤，以提高抗原收率。

B. 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。

C. 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 100µL 碱性洗脱液中加入 Neutralization Buffer（中和液）50µL 或调整 PH 至中性。

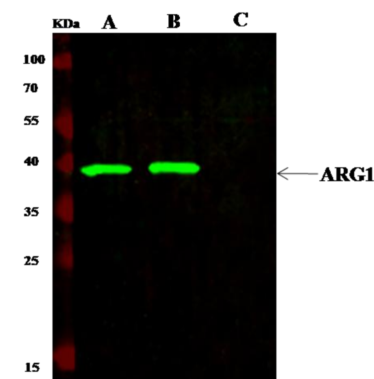
D. Elution Using Sample Buffer（Loading buffer 洗脱）

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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Gel	13% SDS-PAGE reducing gel		
Secondary antibody	Dylight 800-labeled antibody to mouse IgG (H+L), at 1:15000 dilution		
Observed band size	40 kDa		

Reference Information

Related Products

Products	Cat No.
Magnetic Separator-1.5 (2 tubes) for IP	MAGS001
HA Synthetic Peptide	PP100028
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-GFP Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB13105
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 HA-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 HA-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