Protein A/G Magnetic Beads Immunoprecipitation (IP) Kit

Catalog Number: BAG001

Product Contents

Contents	BAG001-20	BAG001-100	Storage
Immunomagnetic Beads Protein A/G[1]	1 mL	5 mL	2-8℃ for 12 months
1×PBST (pH=7.4)	Required but not supplied		
1×PBS (pH=7.4)	Required but not supplied		
NP40 Cell Lysis Buffer[2]	4 mL	20 mL	-20℃ for 12 months
Elution Buffer	1.5 mL	8 mL	2-8℃ for 12 months
Neutralization Buffer	1.5 mL	8 mL	2-8℃ for 12 months

[1] Immunomagnetic Beads Protein A/G (1:1) contains 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 key component, Immunomagnetic Beads Protein A/G is prepared using recombinant Protein A/G (10600-P07E/13103-PNAE, Sino Biological Inc.). The Immunomagnetic Beads Protein A/G is designed for Immunoprecipitation / IP of proteins, protein complex, protein-nucleic acid complex, and other antigens.

Your antibody is added to a tube containing Immunomagnetic Beads Protein A/G. After a short incubation, the antibody's Fc-region binds to the protein A/G.

The tube is then placed on a Magnetic Separator, where the Immunomagnetic Beads migrate to the side wall of the tube facing the Magnetic Separator and allow for easy removal of the supernatant.

The Immunomagneatic Beads-bound antibodies can now be used for IP. Bound material is easily collected using the unique magnetic properties of the Immunomagnetic Beads.

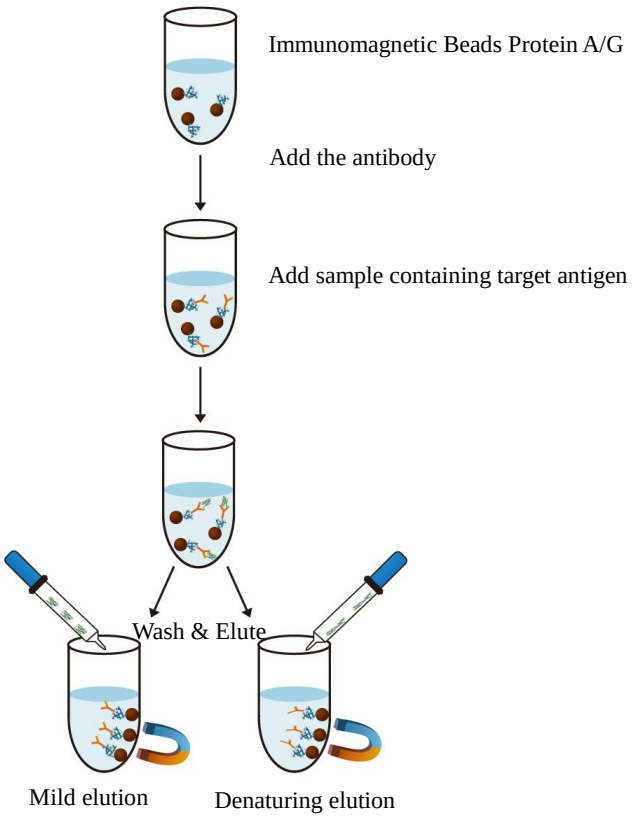

Fig. 1 Immunoprecipitation (IP) Protocol

Protocol

The protocol (Fig. 1) uses 50 µL Immunomagnetic Beads Protein A/G, 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).***

Prepare Beads

1. Resuspend Immunomagnetic Beads in the vial (vortex >30 sec or tilt and rotate 5 min).
2. Transfer 50 µL Immunomagnetic Beads to a tube.
3. Place the tube on the Magnetic Separator to separate the Immunomagnetic Beads from the solution, and remove the supernatant.
4. Remove the tube from the Magnetic Separator.

Bind Antibody

1. Dilute your antibody (typically 1–10 µg) in 200 µL PBST and add to the Immunomagnetic Beads. The amount of antibody used needs to be optimized.
2. Incubate and rotate for 10 min at room temperature.
3. Place the tube on the Magnetic Separator and remove the supernatant.
4. Remove the tube from the Magnetic Separator and wash the Immunomagnetic Beads using 200 µL PBST, by gentle pipetting. For storage of antibody-conjugated Immunomagnetic Beads, we suggest to use PBST to prevent aggregation.

Immunoprecipitate Target Antigen

1. Place the tube (from Bind Antibody step 5) on the Magnetic Separator and remove the supernatant.
2. Add your sample (typically 100–1,000 µL) to the tube and gently mix the Immunomagnetic Beads-antibody complex by gentle pipetting
3. Incubate with rotation for 10 min at room temperature to allow antigen to bind to the Immunomagnetic Beads-antibody complex. Note: it may be necessary to increase incubation times for optimal binding.
4. Place the tube on the Magnetic Separator and remove the supernatant or transfer the supernatant to a clean tube for further analysis, if desired.
5. Wash the Immunomagnetic Beads-antibody-antigen complex 3 times using 200 µL PBS for each wash. Separate on the Magnetic Separator between each wash, remove the supernatant and resuspend the complex by gentle pipetting.
6. Resuspend the above complex in 100 µL PBS and transfer the Immunomagnetic Beads Suspension to a clean tube. This is recommended to avoid co-elution of proteins bound to the tube wall.

Elute Target Antigen

A. Denaturing Elution

1. Place the tube (from step 6 in Immunoprecipitation of Target Antigen) on the Magnetic Separator and remove the supernatant.
2. Add 20 µL Elution Buffer and 5 µL 5×SDS-PAGE Sample Buffer and resuspend the Immunomagnetic Beads complex by gently pipetting.
3. Heat for 5-10 min at 95-100 ℃.
4. Place the tube on the Magnetic Separator and collect the supernatant for further analysis.

B. Mild Elution

1. Place the tube (from step 6 in Immunoprecipitation of Target Antigen) on the Magnetic Separator and remove the supernatant.
2. Add 20 µL Elution Buffer and gently pipette to resuspend the complex. Avoid foaming.
3. Incubate with rotation for 2 min at room temperature to dissociate the complex.
4. Place the tube on the Magnetic Separator and transfer the supernatant containing eluted antibody and antigen to a clean tube. If the eluted protein is to be used for functional assays or stored, the pH of the eluate can be adjusted by adding Neutralization Buffer (about 50 µL of Neutralization Buffer for each 100 µL Elution buffer).

Target Antigen Detection

A. Denaturing Elution

1. SDS-PAGE for staining and protein identification
2. SDS-PAGE for Western blotting
3. SDS-PAGE for Fluorography

B. Mild Elution

1. Protein characterization
2. Immunization
3. Enzyme studies
4. A sequence determination
5. Crystallization

C. No Elution

1. Protein interaction
2. Enzyme studies
3. Bioassays
4. Immunoassays

Protocol 操作步骤

如图 1 所示，取 50 µL protein A/G 免疫磁珠（注：实际去用量根据实际需要可适当增减）。

Cell Lysis（细胞裂解液制备）

细胞裂解液制备可参考标准步骤，我们***建议采用本试剂盒提供的NP40细胞裂解试剂***（注：如有需要可添加蛋白酶抑制剂如：PMSF 1mM 等）。

Prepare Beads（磁珠准备）

1. 使用前，先将磁珠混匀（颠倒 30 秒或者旋涡振荡器混匀）。
2. 取 50 µL 免疫磁珠放入 1.5 mL 离心管。
3. 将上述离心管放入磁力架，待免疫磁珠贴壁后吸去上清。

Bind Antibody（结合抗体）

1. 用 200 µL PBST 稀释 1–10 µg 抗体，稀释后加入到装有 protein A/G 免疫磁珠的离心管中（注：实际抗体使用量需根据实验需要摸索）。
2. 将上述混合物室温孵育 10 分钟左右。
3. 将装有免疫磁珠和抗体的的离心管放入磁力架，待免疫磁珠完全贴壁，吸去上清。
4. 再用 200 µL PBST 洗涤磁珠 3 次。

Immunoprecipitate Target Antigen（沉淀抗原）

1.向上一步装有免疫磁珠的离心管中加入制备好的细胞裂解液 100–1,000 µL，用移液器吹打混匀。
2. 37℃ 孵育 10-15 分钟，使抗原和结合有抗体的免疫磁珠结合（注：为使结合更充分，孵育时间和温度可根据实际需要调整）。
3. 将上一步离心管和混合物放入磁力架，吸取上清（上清可丢弃也可以保存做后续分析）。
4. 取 200 µL PBS 洗涤免疫磁珠-抗体-抗原复合物 3 次。
5. 洗涤完成后用 100 µL PBS 重悬磁珠，用于下一步操作。

Elute Target Antigen（抗原洗脱）

A. Denaturing Elution（变性洗脱）
1. 将装有免疫磁珠复合物的离心管放入磁力架，待磁珠贴壁完全后吸去上清。
2. 向上述离心管中加入 20 µL Elution Buffer（洗脱液）和 5 µL 5×SDS-PAGE Loading Buffer 再用移液器重悬复合物。
3. 95-100 ℃煮沸 5-10 分钟。
4. 将煮沸后的离心管放入磁力架取上清做后续分析（注：上清中含有抗原勿丢弃）。

B. Mild Elution（温和洗脱）
1. 将沉淀抗原第 5 步中的免疫磁珠复合物放入磁力架，待磁珠贴壁后吸去上清。
2. 向上步离心管中加入约 20 µL Elution Buffer（洗脱液），用移液器轻轻混匀避免气泡产生，室温孵育 2 分钟。
4. 将上述离心管放入磁力架，待免疫磁珠贴壁后收集上清（注：上清中含有抗原勿丢弃）。

5. Neutralization（中和）

上述步骤收集上清中抗原，若需做功能验证需加入中和液中和洗脱液（例：100µL 洗脱液加入 50 µL 中和液即可）。

Target Antigen Detection（沉淀后抗原分析）

A. Denaturing Elution（变性洗脱抗原适用方向）
1. 蛋白染色鉴定
2. Western blotting
3. SDS-PAGE for Fluorography

B. Mild Elution（温和洗脱抗原适用方向）
1. 蛋白特征分析
2. 免疫
3. 酶学研究
4. 氨基酸序列分析
5. 蛋白晶体结构分析

C. No Elution（未做洗脱抗原适用方向）
1. 蛋白相互作用
2. 酶学研究
3. 生物分析
4. 免疫学分析

Reference Information

Related Products

Products	Cat No.
Magnetic Separator-1.5 (2 tubes) for IP	MAGS001
Beads Protein A Magnetic Beads Immunoprecipitation (IP) Kit	BA10600
Protein G Magnetic Beads Immunoprecipitation (IP) Kit	BG13103
Protein L Magnetic Beads Immunoprecipitation (IP) Kit	BL11044
Anti-MYC Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB100029
Anti-GFP Tag Magnetic Beads Immunoprecipitation (IP) Kit	TB13105
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

Binding Characteristics of Protein A/G/L

Native Protein A/G/L differ in their binding to Igs from different species and subclasses. For example, human IgG3 will bind strongly to Protein G, but weakly to Protein A.

Species		Protein A	Protein G	Protein L
Human	IgG	+++	+++	+++
	IgG1	++++	++++	++++
	IgG2	++++	++++	++++
	IgG3	-	+++	+++
	IgG4	++++	++++	++++
Rabbit	IgA	+	-	+++
	IgA1	+	-	+++
	IgA2	+	-	+++
	IgD	-	-	+++
	IgE	++	-	+++
	IgM	+	-	+++
	IgG	+++	+++	+
Cow	IgG	+	+++	-
	IgG1	+	+++	-
	IgG2	+++	+++	-
Cat	IgG	+++	+	?
Horse	IgG	++	++++	?
Goat	IgG	+	++	-
	IgG1	+	+++	-
	IgG2	+++	+++	-
Guinea-pig	IgG1	++	+	?
	IgG2	++	+	?
Sheep	IgG	+	++	-
	IgG1	+	++	-
	IgG2	+++	+++	-
Dog	total Ig	++	+	?
Pig	total Ig	+++	++	+++
Rat	IgG	+	++	+++
	IgG1	-	+	+++
	IgG2a	-	++++	+++
	IgG2b	-	++	+++
	IgG2c	++	++	+++
	IgG3	+	++	?
Mouse	IgG	++	++	+++
	IgG1	+	++++	+++
	IgG2a	++++	++++	+++
	IgG2b	+++	+++	+++
	IgG3	++	+++	+++
	IgM	-	-	+++
Chicken	IgY	-	-	-
Monkey(rhesus)	IgG	++++	++++	?
Hamster		+	++	+++
Koala		-	+	?
Llama		-	+	?

Strong binding ＋＋, medium interaction ＋, weak or no interaction －.