Magpoins™ His-Tag Immunoprecipitation KIT

Catalog Number: TBN001

Product	TBN001-20	TBN001-100	Storage
Magpoins™ His-Tag	1 mL	5 mL	4 ℃, 12 month
NP40 cell lysate buffer	4 mL	20 mL	4 ℃, 12 month
2×Binding/Wash Buffer	30 mL	(8×) 20 mL	4 ℃, 12 month
His Elution Buffer	3 mL	15 mL	4 ℃, 12 month
2×Pull-down Buffer	30 mL	(8×) 20 mL	4 ℃, 12 month
Magnetic Separator	1 each	1 each	RT

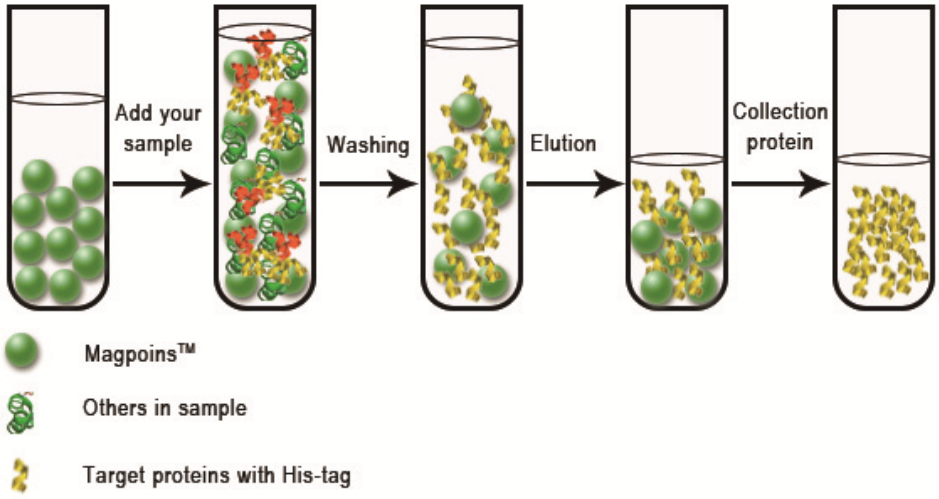
Magpoins™ His-Tag Immunoprecipitation Kit contains 40 mg beads/mL in 20% ethanol and has a capacity of 2mg/mL his-tag protein (28kDa).

Product Description

Magpoins™ His-tag IP KIT was developed for the isolation of his-tag proteins. These Magpoins™ are coated in a nickel-based Immobilized Metal beads. The priority of beads bind his tag proteins is higher selectivity compared to Agarose and Sepharose based bead systems.

Magpoins™ makes the purification of protein more quickly and easily:

Add the beads to a sample containing his-tag proteins and allow the proteins to bind to the Magpoins™. Isolated proteins can be left on the beads and used directly in downstream applications. Alternatively, the isolated his-tag protein can be eluted from the beads. The elution conditions are less stringent than other technologies thus yielding more functional isolated proteins. These characteristics make Magpoins™ His-Tag Immunoprecipitation Kit the ideal product for purifying his-tag proteins expressed in E. coli, mammalian cell, yeast and so on.



Preparation of cell lysates

There are many different ways for preparing a cell lysate containing expressed his-tag proteins. It is important that the lysate does not contain EDTA (or other chelators), ionic detergents, DTT or DTE. A pH between 7 and 8 should be used.

a. The NP40 cell lysis buffer for mammalian and insect cells (supplied with this KIT). See the cell lysis protocol on page 3.

b. French press

c. Sonication

Efficiency of lysis can be increased by the addition of lysozyme (To avoid a sticky pellet, add SuperNuclease (Cat#: SSNP01) In order to avoid protein degradation add Power Protease Inhibitor.

We recommend using the standard protocol for different lysate methods.

Immunoprecipitation Protocol

Prepare your sample containing his-tag protein. Preferentially, you may prepare your sample in a total volume of 700 µL 1×Binding/Wash Buffer.

1. Thoroughly resuspend the Magpoins™ in a tube (vortex >30 sec or tilt and rotate 5 min).
2. Transfer 50 µL (2 mg) Magpoins™ to a micro-centrifuge tube. Place the tube on a magnet separator (Cat#: MAGS001) for 1 min. Aspirate and discard the supernatant. Add your sample (prepared in Binding/Wash Buffer) to beads, Mix well.
3. Incubate on a roller for 5 min at room temperature (or colder if the protein is unstable at room temperature). The incubation time may be increased with the incubate temperature lowing.
4. Place the tube on the magnet separator for 1 min, then discard the supernatant.
5. Wash the beads 4 times with 300 µL Binding/Wash Buffer by placing the tube on a magnet separator for 1 min and discard the supernatant. Resuspend the Magpoins™ thoroughly between each washing step.
6. If the protein is to be eluted, proceed to step 7. If you wish to continue with a Pull-down, then continue to step 1 in “Protein Pull-down”.
7. Add 100 µL His-Elution Buffer. Incubate the suspension on a roller for 5 min at room temperature (or colder if the protein is unstable at room temperature).
8. Put on the magnet for 1 min and transfer the supernatant containing the eluted histidine-tagged protein to a clean tube.

Protein Pull-down

1. Prepare your sample in Pull-down buffer (350 µL) in a total volume of up to 700 µL.
2. Add your sample (prepared in Pull-down Buffer) to the bead/protein complex from step 5 in "Immunoprecipitation Protocol".
3. Incubate on a roller for 10 min at room temperature (or cold if the protein is unstable at room temperature). The incubation time may be increased with the temperature lowing.
4. Place the tube on a magnet separator for 1 min, then discard the supernatant.
5. Wash the beads 4 times with 300 µL Binding/Wash Buffer by placing the tube on a magnet separator for 1 min and discard the supernatant. Resuspend the Magpoins™ thoroughly between each washing step.
6. Add 100 µL His-Elution Buffer. Incubate the suspension on a roller for 5 min at room temperature (or cold if protein is unstable at room temperature).
7. Collect the Magpoins™ at the tube wall using a magnet separator and transfer the supernatant containing the eluted his-tag protein and its interacting protein to a clean tube. The elution volume may be decreased to 50 µL.

Cell Lysis Protocol

A：Lysing Cells Grown as Monolayer Cultures

1. Discard the culture medium. Wash cells twice with ice-cold PBS. Place the culture dishes on ice.
2. Add 1.0 mL of the NP40 buffer of choice (pre-cold to 4 ℃) per 100 mm dish.
For culture dishes of other sizes, adjust the volume of lysis buffer accordingly.
3. Incubate the cells for 10-30 min (depending on the cell line being studied) on ice. Rock the dishes occasionally.
4. Collecting cells in a tube on the bed of ice, allowing the NP40 buffer to drain to one side. Remove the lysate with a pipette. Transfer to a micro-centrifuge tube (or other suitable centrifuge tube). Repeat with each of the remaining dishes.
5. Centrifuge the lysate at 12,000 g for 10 min at 4 ℃.
6. Carefully remove the supernatant to a fresh tube, making sure not to disturb the pellet. Store the lysate at -20℃ until it is needed.

B: Lysing Cells Grown in Suspension

1. Harvest the cells by centrifugation at 480 g for 10 min. Decant and discard the supernatant.
2. Carefully, wash the cell pellet twice with ice-cold PBS. Place the washed cell pellet on ice.
3. Resuspend the pellet in 1.0 mL of the NP40 buffer of choice (pre-cold to 4 ℃) per 1×10^7 to 5×10^7 cells.
4. Incubate the cells for 15 min on ice, vortex the tube occasionally.
5. Centrifuge the lysate at 12,000 g for 10 min at 4 ℃.
6. Carefully, remove the supernatant to a fresh tube, making sure not to disturb the pellet. Store the lysate at -20 ℃ until it is needed.

Magpoins™ His 标签蛋白免疫沉淀试剂盒

货号: TBN001

细胞裂解液制备 Cell Lysis Protocol

我们建议细胞裂解 buffer 中不能含有 EDTA、DTT、 DTE，且 PH 为 7-8 较为合适。本试剂盒中提供了 NP40 裂解 buffer，其它细胞和组织样品可选的裂解方法有：弗氏破碎器，超声破碎等（如有必要，需加入蛋白酶抑制剂）。

A：贴壁细胞裂解液制备

1. 弃培养基，用预冷的 PBS 清洗细胞两次，置于冰上。
2. 用细胞刮刮取细胞，倾斜细胞培养皿吸取细胞并转移至合适大小离心管中。
3. 向收集好的细胞中加入 1.0 mL NP40 细胞裂解 buffer 冰上孵育 10-30 min（中间晃动使裂解 buffer 和细胞充分接触）。
4. 装有裂解液的离心管置于离心机 4 ℃，12,000 g 离心 10 min。
5. 收集上清，并转移至新的离心管-20 ℃保存，备用。

B：悬浮细胞裂解液制备

1. 收集细胞置于合适的离心管中 480×g 离心 10 min 弃上清。
2. 按照上述离心速度和时间用预冷的 PBS 洗涤细胞 3 次，置于冰上。
3. 按照每 1×10^7- 5×10^7 个细胞用 1.0 mL 预冷至 4 ℃ 的 NP40 裂解 buffer 重悬细胞。
4. 上述细胞在冰上孵育 15 min 期间进行混匀使裂解 buffer 和细胞充分接触。
5. 将装有裂解液的离心管放入离心机 12,000×g 4 ℃ 离心 10 min。
6. 收集上清置于新的离心管中，-20 ℃ 保存备用。

免疫沉淀步骤

取细胞裂解液 350 μL 加入 350 μL 2×Binding/Wash Buffer

1. 取用前先将磁珠置于混合仪上混匀。

2. 取 50 μL（2 mg）Magpoins™ 磁珠置于 1.5 mL 离心管中，将离心管置于磁力架中 1 分钟弃去上清，然后移去磁力架，再加入适量（约 700 μL）细胞裂解液和磁珠充分混匀。

3. 磁珠和细胞裂解液室温孵育 5 分钟（若孵育温度降低，则孵育时间需适当延长，如：4 ℃ 可孵育过夜）。

4. 将装有磁珠细胞裂解液的离心管放入磁力架 1 分钟，弃去上清。

5. 每次用 300 μL 1×（或 2×）Binding/Wash Buffer 清洗磁珠 4 次，每次加入 1×（或 2×）Binding/Wash Buffer 后混匀放入磁力架 1 分钟后弃去上清，收集磁珠 his 标签蛋白复合物。

6. 如需洗脱 his 标签蛋白则参照洗脱步骤——第 7 步。如需进行 Pull-down 实验则参照 Protein Pull-down 步骤）。

7. 向上述第 5 步的磁珠中，加入 100 μL His 标签洗脱液室温孵育混悬 5 分钟，如孵育温度降低可适当延长孵育时间。

8. 孵育完成将离心管放入磁力架 1 分钟，收集上清（含有 his 标签蛋白，勿丢弃）放入新的离心管，-20 ℃ 保存备用或者进行下一步实验。

Protein Pull-down（蛋白共沉淀）

1. 取细胞裂解液 350 μL 加入 350 μL 2×Pull-down Buffer

2. 将上述样品加入到 his 标签蛋白磁珠复合物中，室温孵育 10 分钟，如果孵育温度降低可适当延长孵育时间。

3. 孵育完成后将离心管放入磁力架 1 分钟，弃去上清。

4. 每次用 300 μL 1×（或 2×）Binding/Wash Buffer 清洗磁珠 4 次，每次加入 1×（或 2×）Binding/Wash Buffer 后混匀放入磁力架 1 分钟后弃去上清，收集磁珠-his 标签-蛋白-未知蛋白复合物。

5. 向上述第 5 步的磁珠中，加入 100 μL His 标签洗脱液室温孵育混悬 5 分钟，如孵育温度降低可适当延长孵育时间，his 标签洗脱液可以减少至 50 μL。

6. 将离心管放入磁力架，收集上清放入新的离心管，上清中的 his 标签蛋白和未知蛋白复合物可用于后续分析。