Immunoprecipitation protocol (using Precipitor™)

A. Overview

Immunoprecipitation (IP) is the technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that particular protein. This process can be used to isolate and concentrate a particular protein from a sample containing many thousands of different proteins.

Precipitor™ system (Catalog # M0002) is an automated magnetic bead platform for high throughput precipitation and purification of proteins. This machine can handles up to 16 different immunoprecipitation reactions simultaneously by transferring beads from one well to the next for mixing, binding, washing, and elution reactions via the robotic action of parallel magnetic rods. The detail information of the Precipitor™ system can be found on the Abnova's online catalog.

In this guide, we demonstrate a procedure of using Precipitor™ to immunoprecipitate the desire protein (antigen) from a sample by antibody coupled protein A magnetic bead. Figure 1 demonstrates step by step of the precipitation reaction in Precipitor™.

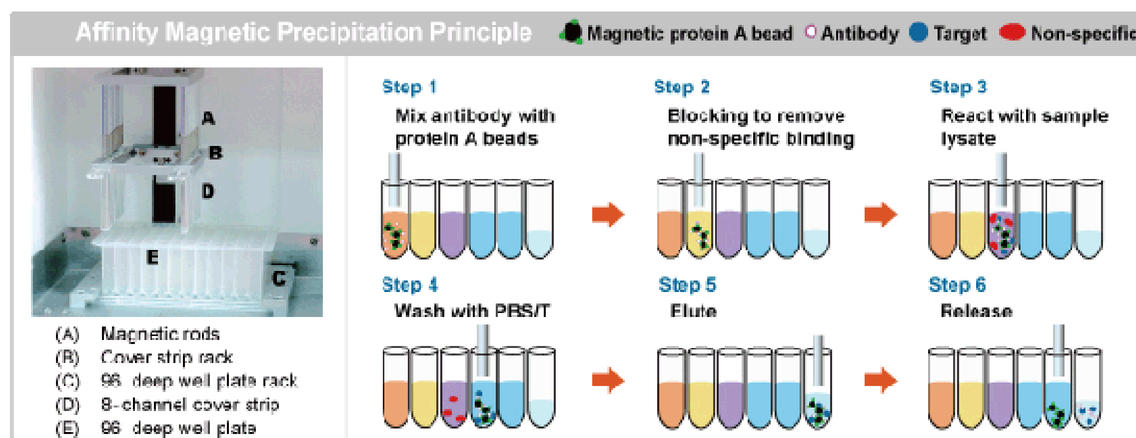

Figure 1. The principle of the precipitation reaction in Precipitor™.

Precipitor™ process one 96 deep well plate each time, and each reaction utilize 6 wells, hence the maximum reaction can be processed by Precipitor™ is 16 immunoprecipitation reactions, each individual reaction area in 96 deep well plate is marked with an black open square in figure 2.

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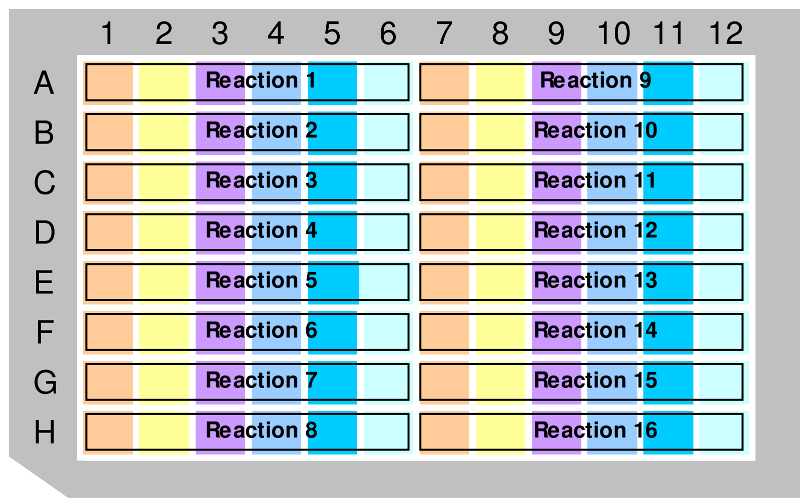

Figure 2. A 96 deep well plate and its reaction areas.

B. Reagents & instruments

- Protein A Magnetic bead (Cat. # U0007)
- Antibody that specific to particular antigen
- Sample(s) that contain this particular antigen: cell/tissue lysate, cell culture supernatant and etc.
- 20x PBS stock (1 L)

NaCl	151.8 g
NaOH	7.0 g
NaH_2PO_4	27.6 g

Weight the salts above to dissolve in 800 ml ddH_2O, adjust pH to 7.0 with NaOH, and fill up to 1000 ml with ddH_2O.

- 0.2% PBST

PBS with 0.2 %(v/v) Tween-20

- Blocking Buffer

Weight 50 g skimmed milk power to 800 ml 0.2%PBST and stir until completely dissolve, and fill up the volume to 1000 ml by adding extra 0.2%PBST.

- 1x Elution Buffer

0.2 M Glycine, 0.15 M NaCl, pH 2.5

- 5x SDS sample buffer

Add 10 g SDS, 0.05 g bromophenol blue, 10.4 ml 14.3 M β-mercaptoethanol in 25 ml 1 M

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Tris-HCl, pH 6.8, adjust the volume to 50 ml with ddH_2O, and finally add 50 ml 100% glycerol, mix well, place at 4℃ for short term storage or -20℃ for long term storage. Take appropriate amount and dilute with ddH_2O to 1x fold before use.

- 96 deep well plate (round bottom) (Cat # U0014)
- 8-channel cover Strip (Cat # U0015)
- Precipitor™ (Catalog #: M0002)

Please read the user manual before using.

C. Assay procedure

- The protocol provide here is just a guide, each user should determine the optimal condition for their own experiment.
- The demonstration protocol below is a single immunoprecipitation reaction run in wells A1 to A6, and the precipitated protein is used in subsequent Western Blot assay.
- Reaction volume of each well should not exceed 1 ml, or the solution may spill out and contaminate the adjacent wells during machine running. The reaction volume used in this protocol is 600 ul.

1. Add X ul antibody and Y ul (50 ug/ul) protein A magnetic beads to A1.
   The recommended molarity ratio between protein A magnetic beads (Cat. # U0007) and antibody is 1:50 ~ 1:100.
2. Add Z ul 0.2% PBST to A1.
   Z ul = 600 ul – (X ul + Y ul)
3. Add 600 ul blocking buffer to well A2 .
4. Add 200 ul sample containing the target antigen to A3, and then add 400ul 0.2% PBST.
5. Add 600 ul 0.2% PBST to A4 and A5 for washing purpose.
6. Add 70ul 1x sample buffer to A6.
7. Insert the 96 deep well plate into the **96 deep well plate rack** (please refer to figure 1).
8. Insert 8-channel cover Strips into the **cover strip rack** (please refer to figure 1).
9. Execute the preset program saved in Precipitor™, the detail setting of the reaction steps is list in table 1.
10. After the machine complete all steps, place the plate on a magnetic separator and collected the supernatant (contain the precipitated protein) in A6.
11. Boil the solution at 100℃ for 5 min for subsequent Western Blot analysis.

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Note: If the precipitated protein will be used in other assay instead of western blot, then replace the 1x SDS sample buffer use in the step 6 with Elution buffer, and the supernatant collected in step 10 should be neutralized by adding Neutralization Buffer (1 M Tris, pH 9.0) (use 2.5 ul of Neutralization buffer for every 50 ul of elution buffer).

Table 1. The immunoprecipitation program for the demonstration protocol above.

Step	Well	Mixing (M)	Collect (S)	Rod	Mixing Speed	Volume	Pause	Vapor (M)
1	1	30	60	ON	Medium	500	OFF	0
2	2	15	60	ON	Medium	500	OFF	0
3	3	30	60	ON	Medium	500	OFF	0
4	4	5	60	ON	Medium	500	OFF	0
5	5	5	60	ON	Medium	500	OFF	0
6	6	30	100	ON	Medium	100	OFF	0
7	5	1	0	OFF	Medium	500	OFF	0
8	0	0	0	OFF	Medium	0	OFF	0

Note: Please read the user manual of the machine carefully before setting the program.

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