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Product Information

Product Name: Micro Spin Filter (IP& Co-IP)
Catalog no.: IP111S
Size: 50 prep

Related products:	Catalog No.
DNA mini-spin columns (30-50µl vol.)	EZC101
DNA micro-spin columns (5-20µl vol.)	EZC106
DNA gel extraction kit	EZGE301

Description:

Micro Spin Filter provides an easy and efficient column method for Immunoprecipitation (IP), Co-IP, and Immunodepletion. All the procedures involve 1) incubation of an antibody with a sample that contains the protein antigen of interest, 2) capturing of the antibody: antigen complex to immobilized Protein A or Protein G agrose beads, 3) washing to remove unbound components of the sample, and 4) separation of antigen and antibody from beads. Here is the advantage by using spin filter.

Traditional method: The entire procedure is performed in a microcentrifuge tube, which requires a) carefully removing of solution from agarose resin after pelted by centrifugation and b) eluting IP-complex by boiling the resin in denaturing sample loading buffer for SDS-PAGE analysis, which will denature and separate antibody into heavy and light subunits.

Column method: Solution can be easily separate from the beads during the wash steps and the antibody: antigen complex can be eluted by low pH buffer and neutralized for subsequent analysis instead of being denatured or cleaved into separate subunits. Furthermore, the resin often can be reused to save the cost.

Contents:

Elution Buffer (Low pH):	2ml
Neutralization Buffer:	0.3ml
5X Sample Loading Buffer (non-reducing):	1.2ml
Spin Filters:	50
Collection tubes:	50

Solutions Recipes:

Elution Buffer: 0.1M glycine, pH 2.0.
Neutralization Buffer: 1M Tris, pH 9.5
Sample Loading Buffer, Non-reducing, (5X): 125mM Tris•HCl (pH6.8), 2.5% SDS, 25% glycerol, lane marker tracking dye (eg, 0.1%BPB)
IP Wash Buffer*: 25mM Tris, 0.15M NaCl, 1mM EDTA, 1% NP-40, 5% glycerol, pH 7.4.
***** This buffer is not included in the kit. You can use any standard IP Lysis/Wash buffers that used in your lab or modify the solution recipes for your application

Storage:

Room temperature

Protocol

1. **Preparation of Immune Complex:** You can prepare the complex by following your lab protocol. Here is the example:
   a. Combine 2-10µg of antibody with 0.5-1mg antigen (clear cell lysate or other sources) in a microcentrifuge tube. Bring up the volume to 50-100µl with IP

wash buffer (you can use any standard IP Lysis/Wash buffers that used in your lab).

b. Incubate the mixture at 4ºC for 1 hour to overnight to form the immune complex.

2. Capture of the Immune Complex:

c. Mix the protein A or G resin by gently swirling the bottle.
d. Add 10-20ul of the resin slurry into the micro spin filter by using a wild-bore or cut pipette tip.
e. Spin the filter at low speed (1000xg) for 1 minute in a microcentrifuge and discard the flow-through.
f. Wash resin twice with 50-100µl of cold IP wash buffer.
g. Remove the excess liquid by gently tap the bottom of the spin filter on a paper towel and put an end cap at the filter bottom.
h. Add the Immune Complex to the protein A or G resin in the spin filter. Close the cap of the collection tube and add end cap* at the bottom of the column, and then incubate the tube with shake for 1 hour.
   * End cap is an option to use since the filter membrane in the column is hydrophobic and the aqua base solution can only go through the filter by centrifugation.
i. Open both top and end caps and place the filter back to the collection tube.
j. Centrifuge at low speed (x1000g) and save the flow-through for analysis.
k. Wash the filter at least two times with 200µl of IP wash buffer.

3. Elution of the Immune Complex: Note: Two options can be used for different application.

Loading Buffer Elution: This step involves heating the sample at 100ºC for Western Blot analysis and the protein A or G beads cannot be reused after heating.

a. Prepare 30-50µl of 2X reducing sample loading buffer (containing 100mM DTT)
   Example: 20µl5x non-reducing sample buffer+25µlddH2O+5µl 1M DTT
   Note: you can also use β-mercaptoethanol (2x concentration: 143mM) to replace 100mM DTT. Example: 20µl5x non-reducing sample buffer + 25µlddH2O +2.5µl βme
b. Add 30-50µl of 2x reducing sample loading buffer to the column. Remove the end cap and keep the column in a new collection tube. Heat the tube at 100ºC for 5-10 minutes.
c. Centrifuge to collect elute and cool the sample before loading to a SDS PAGE gel for analysis.

Low pH Buffer Elution: If you will use the samples for enzymatic or function assay or plan to reuse the beads, this will be the right protocol to follow.

a. Add 10-30µl of low pH elution buffer to the filter and incubate at room temperature for 10 minute.
b. Add 1-3µl of neutralization buffer to the collection tube if needed.
c. Place the filer to the collection tube and centrifuge to collect the flow-through. Perform additional elution as needed.
d. Samples are ready for further analysis.