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Exosome Immunoprecipitation (Protein A)
Exosome Immunoprecipitation (Protein G)

Rev. Date: June 2012 (Rev. 000)

Catalog Numbers 10610D, 10612D

Store at 2˚C to 8˚C

Product Description

Exosome Immunoprecipitation (Protein A) and Exosome Immunoprecipitation (Protein G) are designed for immunoprecipitation of proteins, protein complexes, protein-nucleic acid complexes, and other exosome-related antigens. Antibody (Ab) is added to the protein A- or protein G-coated Dynabeads® suspension. During a short incubation, the Ab binds to the beads via their fragment crystallizable (Fc) region. The Ab-coated beads are separated from the non-bound antibodies using a magnet, and the beads are now ready to be used for immunoprecipitation (IP) (fig. 1).

Product Contents

Product contents	Cat. no.	Volume
Exosome Immunoprecipitation (Protein A)	10610D	1 mL
Exosome Immunoprecipitation (Protein G)	10612D	1 mL

Note: This manual describes the protocols for both Exosome Immunoprecipitation (Protein A) and Exosome Immunoprecipitation (Protein G), but only one of the products will be required. For information of what product is best suited for your needs, see Table 1.

Exosome Immunoprecipitation (Protein A) and Exosome Immunoprecipitation (Protein G) contain 30 mg Dynabeads® suspension/mL in phosphate buffered saline (PBS), pH 7.4, with 0.01% Tween®-20 and 0.09% sodium azide as a preservative.

Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

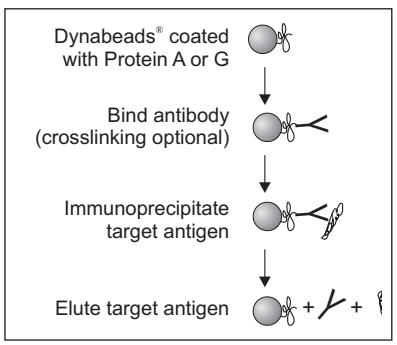

Figure 1: Principle of immunoprecipitation of antigen using Exosome Immunoprecipitation (Protein A) or Exosome Immunoprecipitation (Protein G).

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).

Recommended Buffers

- PBS, pH 7.4 with and without 0.02% Tween®-20.
- Elution Buffer: 50 mM glycine, pH 2.8 or NuPAGE® LDS Sample Buffer, and NuPAGE® Sample Reducing Agent.
- Lysis Buffer: (See "Exosome Lysis" section for details).

General Guidelines

- Both products have a binding capacity of approximately 8 µg human IgG per mg beads. The amount of Ab captured depends on the concentration of Ab and the beads in the starting sample (see Table 1).
- The choice of primary antibody is the most important factor for successful target antigen capture. Note that some antibodies may show reduced antigen-binding efficiency for immunoprecipitation, even though the antibody shows good results in other immunological assays.
- For standard IP use PBS-buffer for antibody binding and washing steps. However, PBS may be substituted by other buffers of choice, such as alternative phosphate buffers, lysis buffer (e.g. RIPA, NP-40), HEPES, or Tris. The recommended Elution Buffer may also be substituted by alternative low pH-, high pH- or high-salt buffers, depending on the application.
- Low-affinity antibodies require increased incubation time, thus it might be beneficial to pre-incubate sample and antibody prior to bead capture. This improves binding kinetics for the antibody and minimizes non-specific binding. This approach is also recommended when working with protein/nucleic acid complexes.
- Increasing incubation times during IP can improve yield when working with low affinity antibodies. Incubation time of 10 min is generally sufficient for most applications, although increasing the incubation time to 15–20 min can increase yield.
  Note: Increased incubation times can lead to higher non-specific binding.
- For sensitive proteins and phosphorylation studies, the isolation protocol including elution may be performed at 2°C to 8°C to avoid protein complex dissociation and minimize enzymatic activity.

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Protocol

This protocol offers a general guideline for IP. Optimization may be required for each antibody and target antigen. The protocol uses 50 µL of Exosome Immunoprecipitation (Protein A) or Exosome Immunoprecipitation (Protein G), but this may be scaled up or down as required.

Exosome Lysis

Exosomes may be lysed using any standard lysis protocol compatible with your sample. The exosome lysis may be optimized using methods based on detergents such as Triton-X-100 or NP-40 [1], RIPA buffer, or precipitation based methods [2, 3].

Prepare Dynabeads®

1. Resuspend the Dynabeads® suspention in the vial (vortex >30 sec or tilt and rotate 5 min).
2. Transfer 50 µL (1.5 mg) beads to a tube.
3. Place the tube on the magnet for 1 min to separate the beads from the solution, and remove the supernatant.
4. Remove the tube from the magnet and proceed directly to “Bind Antibody”.

Bind Antibody

1. Add your antibody (Ab) (typically 1–10 µg) diluted in 200 µL PBS with Tween®-20, to the resuspended beads from step 4 of "Prepare Dynabeads®". The optimal amount of Ab needed depends upon the individual Ab used.
2. Incubate with rotation for 10 min at room temperature.
3. Place the tube on the magnet for 1 min and remove the supernatant.
4. Remove the tube from the magnet and resuspend the beads-Ab complex in 200 µL PBS with Tween®–20. Wash by gentle pipetting.
5. Proceed to "Immunoprecipitate Target Antigen".

For storage of Ab-conjugated Dynabeads® use PBS, pH 7.4 with 0.01–0.1% Tween®-20 to prevent aggregation.

Crosslinking

To avoid co-elution of your antibody, cross-link your antibody to the Dynabeads® before continuing with IP. Use the cross-linking reagent BS3. For further information and procedure, visit www.lifetechnologies.com/crosslinking.

Immunoprecipitate Target Antigen

1. Place the tube from step 5 of "Bind Antibody" on the magnet for 1 min and remove the supernatant.
2. Add your sample containing the antigen (Ag) (typically 100–1000 µL) and gently pipet to resuspend the Dynabeads®-Ab complex.
3. Incubate with rotation for 10 min at room temperature to allow Ag to bind to the Dynabeads®-Ab complex. **Note:** Depending on the affinity of the antibody, it may be necessary to increase incubation times for optimal binding.
4. Place the tube on the magnet for 1 min. Transfer the supernatant to a clean tube for further analysis, if desired.
5. Wash the Dynabeads®-Ab-Ag complex 3 times using 200 µL PBS for each wash. Separate on the magnet between each wash, remove supernatant, and resuspend by gentle pipetting.
6. Resuspend the Dynabeads®-Ab-Ag complex in 100 µL PBS and transfer the bead suspension to a clean tube. This is recommended to avoid co-elution of proteins bound to the tube wall.
7. Proceed to "Elute Target Antigen".

Elute Target Antigen

A. Denaturing elution

1. Place the tube from step 7 of "Immunoprecipitate Target Antigen" on the magnet for 1 min and remove the supernatant.
2. Add 20 µL Elution Buffer or Lysis Buffer, and 10 µL pre-mixed NuPAGE® LDS Sample Buffer and NuPAGE Sample Reducing Agent (mixed as per manufacturer’s instructions).
3. Gently pipet to resuspend the Dynabeads®-Ab-Ag complex.
4. Heat for 10 min at 70 ºC.
5. Place the tube on the magnet for 1 min and load the supernatant/sample onto a gel.

Note: As an alternative, the Dynabeads®-Ab-Ag complex can be resuspended in a sample buffer of your choice (e.g. SDS sample buffer). Follow the recommended temperatures and heating times for these buffers prior to gel loading.

B. Non-denaturing elution

1. Place the tube from step 7 of "Immunoprecipitation of Target Antigen" on the magnet for 1 min and remove the supernatant.
2. Add 20 µL Elution Buffer and gently pipet to resuspend the Dynabeads®-Ab-Ag complex. Avoid foaming.
3. Incubate with rotation for 2 min at room temperature to dissociate the complex.
4. Place the tube on the magnet for 1 min, and transfer the supernatant containing eluted Ab and Ag 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 1 M Tris, pH 7.5.

For storage of the immunoprecipitated protein, freeze the Dynabeads®-Ab-Ag complex after adding the Elution Buffer (step 2 of both elution procedures). For analysis of the sample, thaw and continue with the elution protocol.

Description of Materials

The Exosome Immunoprecipitation (Protein A) and Exosome Immunoprecipitation (Protein G) contain uniform, 2.8 µm, superparamagnetic Dynabeads® coated with recombinant Protein G (approximately 45 kDa) and with recombinant Protein A (approximately 45 kDa) respectively, covalently coupled to the surface.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer® Sample Mixer	15920D
Immunoprecipitation Kit – Dynabeads® Protein A	10006D
Immunoprecipitation Kit – Dynabeads® Protein G	10007D
Cell Extraction Buffer	FNN0011
NP40 Cell Lysis Buffer	FNN0021

REF on labels is the symbol for catalog number.

Table 1: Binding strength of Protein A and Protein G to different species of Ig's and their subclasses.

Species	Ig Subclass	Binding strength to Protein A	Binding strength to Protein G
Human	IgG1, IgG2, IgG4	+++	+++
	IG3	+	+++
	IgD	-	-
	IgD	+	-
	Fab	+	-
	ScF_v	+	-
Mouse	IgG1	+	++
	IgG2a, IgG2b, IgG3	+++	+++
	IgM	-	-
Rat	IgG1	+	++
	IgG2a	-	+++
	IgG2b	-	+
	IgG2c	+++	+++
Goat	IgG1	+	+++
	IgG2	+++	+++
Sheep	IgG1	+	+++
	IgG2	+++	+++
Cow/Bovine	IgG1	+	+++
	IgG2	+++	+++
Horse	IgG(ab)	+	-
	IgG(c)	+	-
	IgG(T)	-	+++
Rabbit	Total Ig	+++	+++
Dog	Total Ig	+++	+
Cat	Total Ig	+++	+
Pig	Total Ig	+++	+
Guinea pig	Total Ig	+++	+
Chicken	Total Ig	-	-

References

1. C. Théry, A. Clayton, S. Amigorena, and G. Raposo (2006) Current Protocols in Cell Biology 3.22.1-3.22.29
2. N. Likhite and U.M. Warawdekar (2011) Journal of Biomolecular Techniques 22: 33-44
3. C. Yong, P. Truman (2012) Analytical Biochemistry 421: 330-332

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