Protein A-coupled Magnetic Beads (used for MPCLIA)

Cat. No. MPC-A001 Size 10 mg / 100 mg (20mg*5)

Description (Background)

The Protein A-coupled Magnetic Beads are 2.8 μm superparamagnetic particles covalently coupled to a highly pure form of Diamond Protein A. The beads can be used to capture the antibodies in Chemiluminescence procedures.
The Diamound Protein A is a 26.5 kDa surface protein, it binds the heavy chain within the Fc region of most immunoglobulins and also within the Fab region in the case of the human VH3 family.
The Protein A-coupled Magnetic Beads is easy to capture the most antibodies, and the bounded antibody have no activity lost, this ready to use products could greatly save your protein coupling time and hassle, and help us get the best performance and highly reproducible results.

Specifications

Items	Details
Detection Method	Chemiluminescence
Product Type	Magnetic Beads (Protein A)
Quantity Size	10 mg / 100 mg
Physical Appearance	lyophilized powder mixture
Particle size	2.8 μm
Beads Surface	Hydrophilic
Amount of Coupled Protein	About 755 pmol (20 μg) Protein A/mg Beads
Binding Capacity	15-25 μg Human antibody or Human IgG Fc tag proteins/mg beads
Emission Wavelength	Measured relative light units (RLU) at 430 nm
Formulation	Lyophilized from 0.22 μm filtered solution in 1×PBS,pH7.4 with 0.1% Tween-20, 0.5% BSA and 10% Trehalose.
Reconstitution	1 mL sterile deionized water to 10 mg size (10 mg beads/mL) 2 mL sterile deionized water to 20 mg size (10 mg beads/mL)
Storage temperature	This product is stable for 1 year when stored at -20°C in lyophilized state. 2-8°C for 1 months under sterile conditions after reconstitution Please avoid more than 3 freeze-thaw cycles
Transport	The product is shipped at ambient temperature.
Note	For research use only

Shipping and Storage

The product is shipped at room temperature.
Upon receipt, please store the product at -20°C or lower away from light.
The product is stable after storage at:
-20°C for 1 years in lyophilized state;

2-8°C for 7 days under sterile conditions after reconstitution. -20°C for longer time.
Please avoid more than 3 freeze-thaw cycles.
Do not use reagents past their expiration date.

Applications

The Protein A-coupled Magnetic Beads is used to capture the most antibodies, it can combination with Acridine ester markers in chemiluminescence technology, The Acridine ester markers such as Streptavidin-Acridine Ester can capture the biotinylated proteins or molecules, this allows detection of antigen and antibody binding or antibody screening.

Application Suggestion

The Protein A-coupled Magnetic Beads can be used in combination with different Acridine ester markers, such as Streptavidin-Acridine Ester or other Acridine ester markers of directly labeled proteins, this allows detection of biotinylated proteins & Any binding Fc tagged proteins, biotinylated antigen & antibodies binding or antibody screening. The paired schemes are shown in the following table:

Protein A-coupled Magnetic Beads can bind with	**Acridine ester markers**	**Acridine ester markers reference**	**Acridine ester markers binding molecules**
Antibodies or Fc tagged protein are captured by Protein A	Streptavidin-Acridine Ester (SA-AE)	ACRO, Cat. No. STN-NA114	Biotinylated proteins or molecules
Antibodies or Fc tagged protein are captured by Protein A	Directly labeled proteins-Acridine Ester	According to your experiment	According to your experiment

General guidelines

1. The Protein A-coupled Magnetic Beads just suit for Antibodies or Fc tagged protein can be captured by Protein A, it can bind the most antibodies.
2. Because the particle size of magnetic beads is only 2.8 μm, beads may stick to the side of the bottle in the shipping process. Before opening, tap the bottle to ensure the beads settle to the bottom of the bottle.
3. It is strongly recommended to reconstitute the Protein A-coupled Magnetic Beads with sterile deionized water to a stock solution of 10 mg/mL, avoid vigorous shaking or vortexing, please reconstitute the protein following the COA.
4. The Protein A-coupled Magnetic Beads should be used together with different Acridine ester markers, select suitable acridine ester markers according to the requirements of the experiment.
5. To decrease background signal, choosing a reasonable experimental condition is very important. Before the formal experiment, an optimization or a pilot test is highly recommended. Optimizing the concentrations of the antigen, antibodies, Acridine ester markers, and Protein A-coupled Magnetic Beads may be required.
6. To limit nonspecific signal due to unsuitable reagent solutions, please choose the most appropriate buffer solution for the experiment. The Assay/Washing Buffer should be IgG free, which will interfere with samples binding to the Protein A.
7. To reduce cross-contamination between positive samples and negative samples, please add samples in the correct way and sequence.
8. If the signal value is not available, check whether the Protein A-coupled Magnetic Beads and other reagent are

expired. Do not use an expired buffer and reagent. The components of different batch should not be mixed used because it may lead to incorrect results.

Materials and Reagents Preparation

The required materials and reagents are prepared according to the below table.

Name	Specifications	Details	Remark
Protein A-coupled Magnetic Beads (used for MPCLIA)	10 mg Beads or 100 mg Beads (20 mg*5)	About 755 pmol (20 μg) Protein A/mg Beads	Reconstitute the Beads with sterile deionized water to 10mg beads/mL
Magnetic separator stand	For 1.5mL, 2mL or 15mL tubes	Under 2000 to 4000 Gs of magnetic field intensity, the beads can be completely attracted to the separator and separation from supernatant within 2 minutes.	If the storage solution or formulation buffer of beads have any interference, please wash the magnetic beads with appropriate washing buffer first, and this time, we need a Magnetic separator.
Acridine ester markers	According to your experiment	-	Such as Streptavidin-Acridine Ester, you can also use a directly acridine ester labeled proteins.
Washing Buffer	1×PBST, pH7.2-7.4	1×PBS, pH 7.3, 0.05% Tween-20	If your sample could be disturbed by BSA, you can omit it. For many applications, adding a detergent such as 0.01–0.1% Tween™ 20 to the Assay/washing buffers could reduce non-specific binding.
Assay Buffer	0.5% BSA in 1×PBST, pH7.2-7.4	0.5g BSA in 100mL 1×PBST	The Buffer often used in serum-free Binding Assays.
Chemiluminescent Substrate Solution	-	Trigger A (Oxidant solution) and Trigger B (Enhancer solution)	Such as Chemiluminescent Substrate Solution (AE Marker) from ACRO, cat. No. ABK-001
Bovine Serum Albumin (IgG-Free, Protease-Free)	IgG-Free, Protease-Free	-	It is recommended to use IgG-Free, and protease-Free BSA, such as Jackson, Cat. No. 001-000-162
Tubes	According to your experiment	-	If no BSA protectant is added to your reaction system, please select low adsorption tubes.
Some other Materials and Reagents	According to your experiment	-	For example, magnetic separation column and Pipette and reagent bottles that comes with your equipment.

General Protocols

1. Magnetic Beads Reconstitution

To make sure the beads entirely removed, you can reconstitute the beads following the COA. For example, when dealing with 10 milligrams of magnetic beads, you can add 1 mL sterile deionized water to the beads to 10 mg Beads/mL.

2. Wash the magnetic beads

When do the chemiluminescence experiment, make sure the storage solution or formulation buffer of beads buffer is suitable for the reaction, if there is any interference, please wash the magnetic beads with appropriate washing buffer first. In most cases, we don't need this bead washing step, if you need this step, please follow the steps below.

1) Place the tube with reconstituted beads on a magnetic separator for 2 min. Remove the supernatant.

2) Remove the tube from the magnetic separator and resuspend the pelleted beads in a reasonable volume of Assay/ Washing Buffer (when you take 100μL of 10mg/mL beads, you need at least 400μL washing buffer to wash the beads each time). Mix by vortex for approximately 10 sec.

3) Place the tube on the magnetic separator for 2 min. Remove the supernatant.

4) Wash the beads for three times in total by repeating steps 2) and 3).

5) Resuspend the Beads to a suitable volume.

Procedure for assay

1. **Prepare materials and tools for your experiment**, such as Protein A-coupled Magnetic Beads, protein or antibodies, Acridine ester markers, Chemiluminescent Substrate Solution, assay buffer, washing buffer, Magnetic Separator and so on.
2. **Prepare the protein**, if the sample protein needs to be reconstructed, please reconstitute the protein following the COA. To avoid surface adsorption loss and inactivation, the reconstituted protein must NOT be aliquoted to less than 10 μg per vial.
3. **Prepare Protein A-coupled Magnetic Beads with target Antibodies or Fc Tagged proteins**
   When you use the Protein A-coupled Magnetic Beads, the antibodies or Fc Tagged proteins can be captured to Protein A on beads. Dilute the Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001) to required concentration (such as 200 μg/mL) with Assay Buffer (such as 0.5% BSA in 1×PBST, pH7.2-7.4), add into Magnetic beads bottle, add 50 μL (10 μg) to each test.
4. **Prepare Acridinium ester markers according to correct experimental procedures**. if you choose an acridine ester marker that directly labeled with protein, please select appropriate labeling conditions to ensure that the protein remains active after labeling, you can also choose Acridinium ester markers that are labeled, such as Streptavidin-Acridine ester.
5. It is recommended to dilute the Acridine ester markers to an appropriate concentration. For example, when you use the Streptavidin-Acridine ester (Cat. No. STN-NA114) to bind biotinylated protein, you can dilute the Streptavidin-Acridine ester to 0.16 μg/mL with Assay Buffer in R2 bottle (Acridine ester bottle), add 50 μL (0.008 μg) to each test.
   If take the antibody or Fc tagged protein as samples, dilute the test sample with the Assay Buffer to a series of concentrations or to a certain dilution ratio. Then add the series of concentration samples to the tests in the system. And meanwhile dilute the biotinylated protein to a reasonable concentration with Assay Buffer in R1 bottle (such as 0.8 μg/mL, add 50 μL (0.04 μg) to each test).
   If take the biotinylated protein as samples, dilute the biotinylated protein with the Assay Buffer to a series of concentrations, and dilute antibody or Fc tagged protein to a reasonable concentration with Assay Buffer, add the samples into the system.
6. Prepare the Chemiluminescent Substrate Solution (AE Marker) (ACRO, Cat. No. ABK-001), take out the equal volume of the Trigger A (Oxidant solution) and Trigger B (Enhancer solution) required for the experiment, and add them to the reagent bottles accompanying the equipment, after the experiment, do not pour the remaining solution back to the original packaging bottle to avoid contamination.

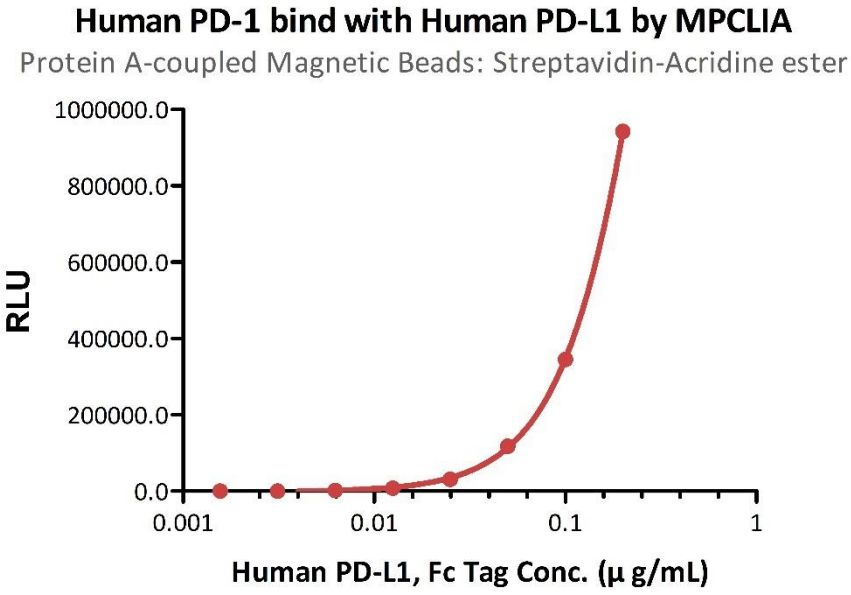
Note: Exposure to the sun or any other intense light can harm the Chemiluminescent Substrate Solution For best results, keep the Substrate Solution in an amber bottle and avoid prolonged exposure to any intense light Short-term exposure to typical laboratory lighting will not harm the Substrate Solution.

7. Get your Chemiluminescence Immunoassay System ready and set up the running program. Confirm equipment readiness. Each instrument is programmed differently, make the correct program settings according to your own equipment design and experimental requirements.
8. Check your program, samples, beads, reagents, buffer and others details, make sure there are no problems and start the program.
9. Add an appropriate volume of Working Solution to each test, such as add 100 µL to each test.
10. Measure the relative light units (RLU, ~430 nm) on your equipment, due to equipment differences, the final read value of relative light units (RLU) may be different, the operator should be familiar with their own equipment program Settings.

Figures

Protein A-coupled Magnetic Beads paired with Streptavidin-Acridine ester:

Beads	**Beads amount**	**Acridine Ester (AE)-Labeled protein**	**AE-Labeled protein amount**	**R1 reagent**
Protein A-coupled Magnetic Beads (Cat. No. MPC-A001)	10 µg Beads /Test	Streptavidin-Acridine ester (Cat. No. STN-NA114)	0.008 µg /Test	Biotinylated Human PD-1, Avitag,His Tag (recommended for biopanning) (Cat. No. PD1-H82E4)
R1 reagent amount	**Sample**	**Sample Conc.**	**sensitivity**	
0.04 µg /Test	Human PD-L1, Fc Tag (Cat. No. PD1-H5258)	0.2-0.00156 µg/mL	3.125 ng/mL	

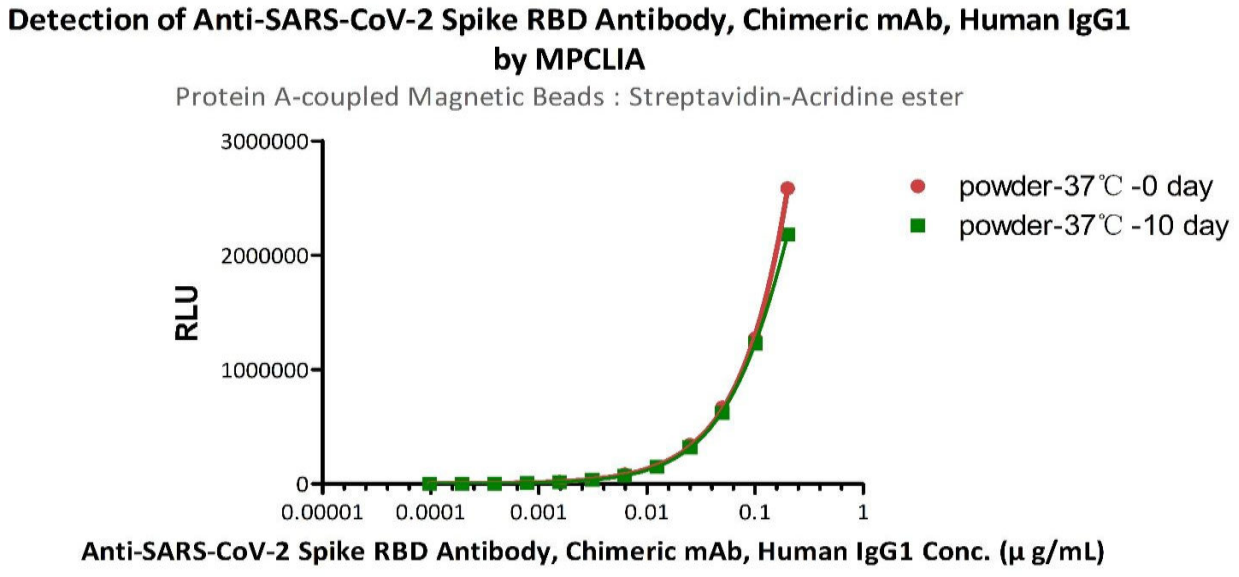


Immobilized 0.04 μg /Test of Biotinylated Human PD-1, Avitag,His Tag (recommended for biopanning) (Cat. No. PD1-H82E4) to the Streptavidin-Acridine ester (Cat. No. STN-NA114, 0.008 μg /Test), incubated with 100 μL /Test of Human PD-L1, Fc Tag (Cat. No. PD1-H5258) at increasing concentration coupled to Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001) (10 μg beads/Test). Detection was performed with sensitivity of 3.125 ng/mL in Magnetism particulate chemiluminescence immunoassay (MPCLIA) (KEYSMILE, SMART 6500S) (QC tested).

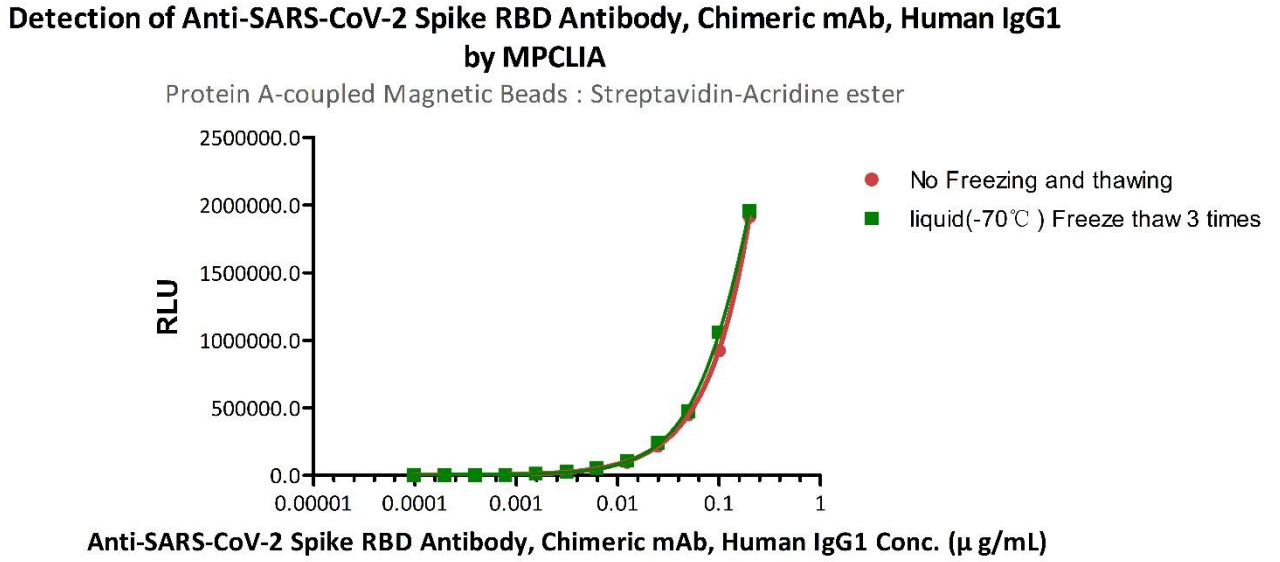
Stability of Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001):

Immobilized 0.04 μg /Test of Biotinylated SARS-CoV-2 Spike RBD, His,Avitag (Cat. No. SPD-C82E9) to the Streptavidin-Acridine ester (Cat. No. STN-NA114, 0.008 μg /Test), incubated with 100 μL /Test of Anti-SARS-CoV-2 Spike RBD Antibody, Chimeric mAb, Human IgG1 (AM122) (Cat. No. S1N-M12A1) at increasing concentration coupled to Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001) (10 μg beads/Test). Detection was performed with sensitivity of 0.098 ng/mL in Magnetism particulate chemiluminescence immunoassay (MPCLIA) (KEYSMILE, SMART 6500S) (Routinely tested).

Beads	**Beads amount**	**Acridine Ester (AE)-Labeled protein**	**AE-Labeled protein amount**	**R1 reagent**
Protein A-coupled Magnetic Beads (Cat. No. MPC-A001)	10 μg Beads /Test	Streptavidin-Acridine ester (Cat. No. STN-NA114)	0.008 μg /Test	Biotinylated SARS-CoV-2 Spike RBD, His,Avitag (Cat. No. SPD-C82E9)
R1 reagent amount	**Sample**	**Sample Conc.**	**sensitivity**	
0.04 μg /Test	Anti-SARS-CoV-2 Spike RBD Antibody, Chimeric mAb, Human IgG1 (AM122) (Cat. No. S1N-M12A1)	0.2-0.000098 μg/mL	0.098 ng/mL	



The Product Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001) is high stability. The accelerated stability of the product within 10 days at 37℃ with no more than 10% performance decrease.



The Product Protein A-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A001) is high stability. After freezing and thawing for 3 times, the activity of the product has no more than 10% performance decrease.

Frequently asked questions (FAQs)

1. What should be paid attention to in the application of Protein A-coupled Magnetic Beads in chemiluminescence immunoassay?

   The Protein A-Magnetic Beads should be used together with different Acridine ester markers such as Streptavidin Acridine ester but not Anti-Mouse IgG or Anti-human IgG-Acridine Ester, the magnetic beads should not bind to Acridine ester markers, this is very important for experimental design to decrease background signal.

   For example, when using Streptavidin Acridine ester to capture biotinylated antigen protein, the Acridine ester markers should not cross-react with Protein A-Magnetic Beads or the antibodies, and the Protein A-Magnetic Beads should only bind to the antibodies.
2. How long can Protein A-Magnetic Beads be used in a system reagent bottle after being diluted into a certain concentration?

   After diluting Protein A- Magnetic Beads to a certain concentration for experiments, it is recommended to use it within one month.
3. What should be attention to when Protein A beads capture antibodies or Fc tagged proteins?

   The Protein A-coupled Magnetic Beads is easy to capture the most human IgG antibodies, weak or nonbinding to some Mouse IgG antibodies or Mouse IgG Fc tagged proteins. Make sure the antibodies can bind to Protein A when using some small species antibodies, otherwise a negative result may be caused by Protein A failing to capture these antibodies.