Anti-His Tag Antibody (Mouse IgG1)-coupled Magnetic Beads

(recommended for MPCLIA)

Cat. No. MPC-A005 Size 10mg / 100mg (20mg*5)

Description (Background)

The Anti-His Tag-coupled Magnetic Beads are 2.8 μm superparamagnetic particles covalently coupled to a highly affinity Monoclonal Mouse Anti-His Tag antibody. The beads can be used to capture the proteins containing N-terminal or C-terminal His Tags (6×His-10×His) in Chemiluminescence procedures.

The Monoclonal Mouse Anti-His Tag antibodies are produced from a hybridoma resulting from fusion of SP2/0 myeloma and B-lymphocytes obtained from a mouse immunized with His Tag Protein. The Anti-His Tag-coupled Magnetic Beads is easy to capture his Tagged protein or other molecules, 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 (Anti-His Tag Antibody)
Quantity Size	10mg / 100 mg
Physical Appearance	lyophilized powder mixture
Particle size	2.8 μm
Beads Surface	Hydrophilic
Amount of Coupled Protein	About 133 pmol (20 μg) Monoclonal Mouse Anti-His Tag antibodies/ mg Beads
Binding Capacity	3-8 μg His 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 10mg size (10 mg beads/mL) 2 mL sterile deionized water to 20mg size (10 mg beads/mL)
Storage temperature	This product is stable for 1 year when stored at -20 ℃. Please avoid more than 3 freeze-thaw cycles. Immediate use after reconstitution is highly recommended.
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 1 month under sterile conditions after reconstitution.
Please avoid more than 3 freeze-thaw cycles.
Do not use reagents past their expiration date.

Applications

The Anti-His Tag-coupled Magnetic Beads is used to capture his Tagged protein or molecules, 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, AHG-Acridine ester can capture human antibodies, this allows detection of ligand / acceptor binding assay, antigen/antibody binding assay, or antibody screening.

Application Suggestion

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

Anti-His Tag-coupled Magnetic Beads can bind with	**Acridine ester markers**	**Acridine ester markers reference**	**Acridine ester markers binding molecules**
His tagged proteins	Streptavidin-Acridine Ester (SA-AE)	ACRO, Cat. No. STN-NA114	Biotinylated proteins or molecules
His tagged proteins	Anti-Human IgG-Acridine ester (AHG-AE)	ACRO, Cat. No. AHG-Y69	Human antibody or Human IgG Fc tag proteins
His tagged proteins	Directly labeled proteins-Acridine Ester	According to your experiment	According to your experiment

General guidelines

1. The Anti-His Tag-coupled Magnetic Beads just suit for His tagged proteins, some other molecules may not be able to bind to the Monoclonal Mouse Anti-His Tag antibody.
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 Anti-His Tag-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 Anti-His Tag-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 Anti-His Tag-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 Anti-Human IgG.
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 Anti-His Tag-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
Anti-His Tag-coupled Magnetic Beads (used for MPCLIA)	10 mg Beads or 100 mg Beads (20 mg*5)	About 133 pmol (20μg) Monoclonal Mouse Anti-His Tag antibodies/ 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, AHG-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 10mg 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 Anti-His Tag-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 Anti-His Tag-coupled Magnetic Beads with target His Tagged proteins**
   When you use the Anti-His Tag-coupled Magnetic Beads, the His Tagged proteins can be captured to Monoclonal Mouse Anti-His Tag antibodies on beads. Dilute the Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A005) 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 Anti-Human IgG-Acridine ester.
5. It is recommended to dilute the Acridine ester markers to an appropriate concentration. For example, when you use the Anti-Human IgG-Acridine ester (Cat. No. AHG-Y69) to bind human antibody or Fc tagged protein, you can dilute the Anti-Human IgG-Acridine ester to 0.8 μg/mL with Assay Buffer in R2 bottle (Acridine ester bottle), add 50 μL (0.04 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 His Tagged protein to a reasonable concentration with Assay Buffer in R1 bottle (such as 0.5 μg/mL, add 50 μL (0.025 μg) to each test).
   If take the His Tagged protein as samples, dilute the His Tagged 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.

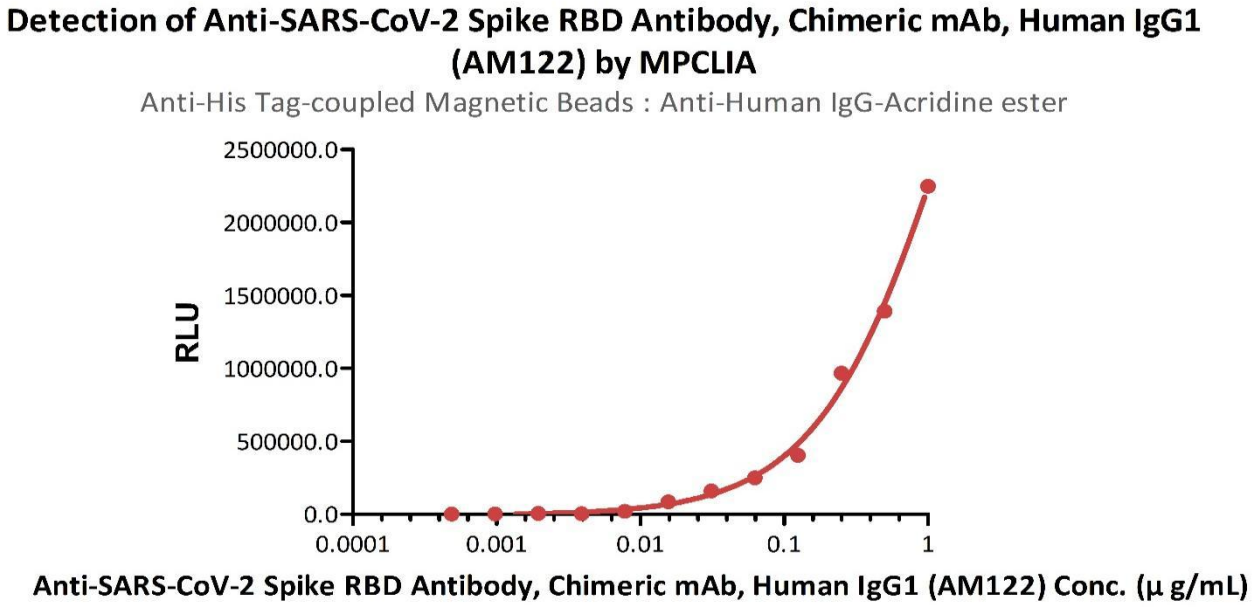
Note: Exposure to the sun or any other intense light can harm the Chemiluminescent Substrate Solution For best results, keep the Substrate Solution in an amber bottle and avoid prolonged exposure to any intense light Short-term exposure to typical laboratory lighting will not harm the Substrate Solution.

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, ~430nm) 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

Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) paired with Anti-Human IgG-Acridine ester :

Beads	**Beads amount**	**Acridine Ester (AE)-Labeled protein**	**AE-Labeled protein amount**	**R1 reagent**
Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A005)	10 µg Beads /Test	Anti-Human IgG-Acridine ester (Cat. No. AHG-Y69)	0.04 µg /Test	SARS-CoV-2 S protein RBD, His Tag (Cat.No. SPD-C52H3)
R1 reagent amount	**Sample**	**Sample Conc.**	**sensitivity**	
0.025 µg /Test	Anti-SARS-CoV-2 Spike RBD Antibody, Chimeric mAb,Human IgG1 (AM122) (Cat. No. S1N-M12A1)	1-0.00049 µg/mL	0.98 ng/mL	

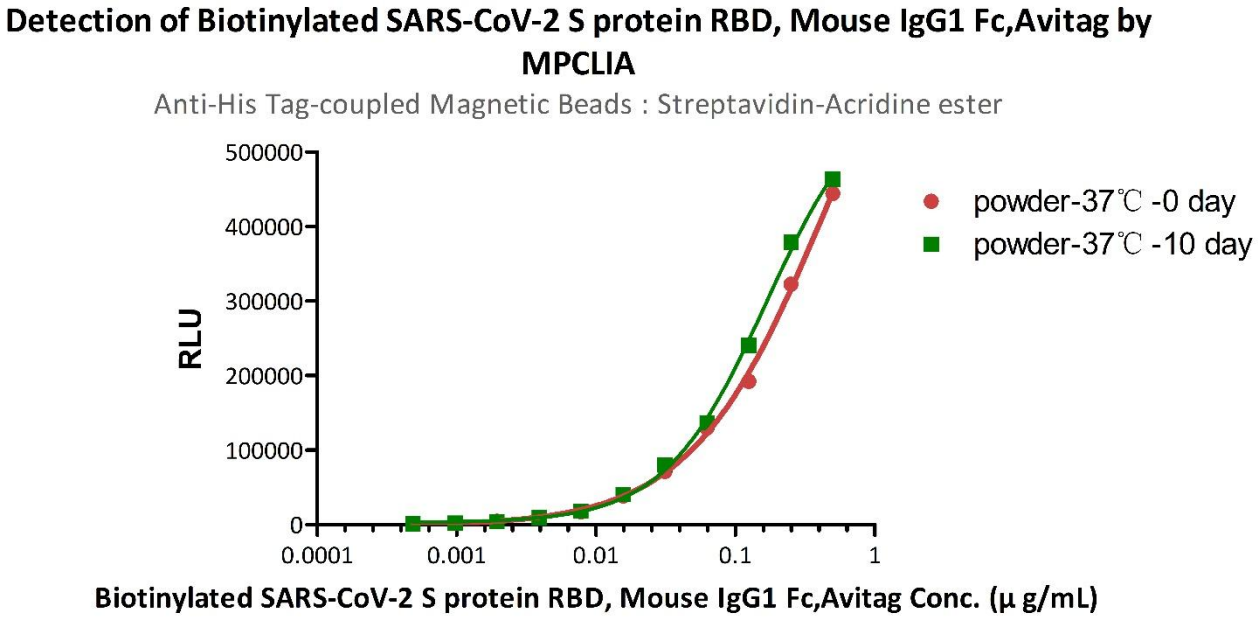


Immobilized 0.025 μg /Test of SARS-CoV-2 S protein RBD, His Tag (Cat. No. SPD-C52H3) to the Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A005) (10 μg beads/Test), incubated with 100 μL /Test of Anti-SARS-CoV-2 Spike RBD Antibody, Chimeric mAb, Human IgG1 (AM122) (Cat. No. S1N-M12A1) at increasing concentration coupled to Anti-Human IgG-Acridine ester (Cat. No. AHG-Y69, 0.04 μg /Test). Detection was performed with sensitivity of 0.98 ng/mL in Magnetism particulate chemiluminescence immunoassay (MPCLIA) (KEYSMILE, SMART 6500S) (QC tested).

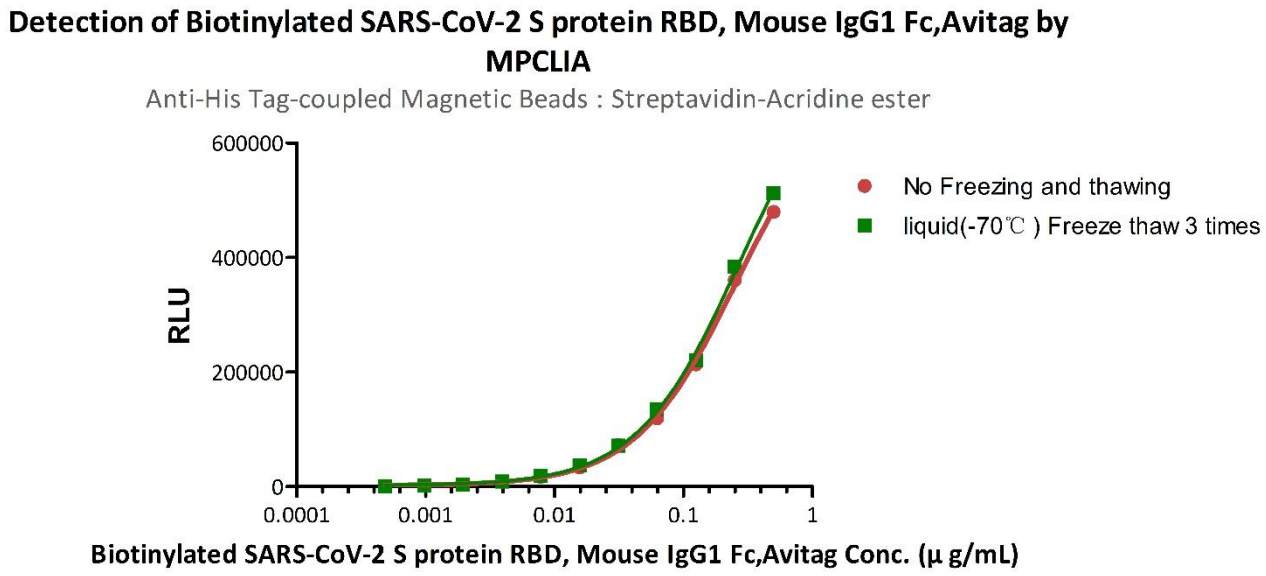
Stability of Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (used for MPCLIA) (Cat. No. MPC-A005):

Immobilized 0.05 μg /Test of Human ACE2, His Tag (Cat. No. AC2-H52H8) to the Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A005) (10 μg beads/Test), incubated with 100 μL /Test of Biotinylated SARS-CoV-2 S protein RBD, Mouse IgG1 Fc,Avitag (Cat. No. SPD-C82Aa) at increasing concentration coupled to Streptavidin-Acridine ester (Cat. No. STN-NA114, 0.02 μg /Test). Detection was performed with sensitivity of 0.49 ng/mL in Magnetism particulate chemiluminescence immunoassay (MPCLIA) (KEYSMILE, SMART 6500S) (Routinely tested).

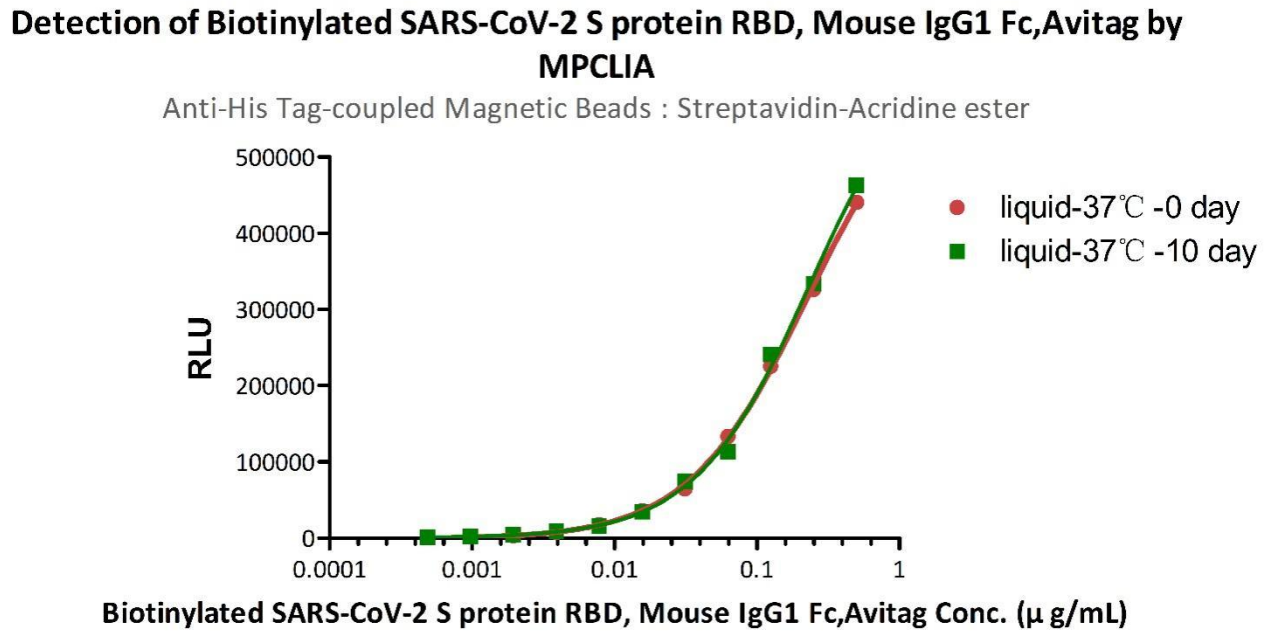
Beads	**Beads amount**	**Acridine Ester (AE)-Labeled protein**	**AE-Labeled protein amount**	**R1 reagent**
Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (Cat. No. MPC-A005)	10 μg Beads /Test	Streptavidin-Acridine ester (Cat. No. STN-NA114)	0.02 μg /Test	Human ACE2, His Tag (Cat. No. AC2-H52H8)
R1 reagent amount	**Sample**	**Sample Conc.**	**sensitivity**	
0.05 μg /Test	Biotinylated SARS-CoV-2 S protein RBD, Mouse IgG1 Fc,Avitag (Cat. No. SPD-C82Aa)	0.5-0.00049 μg/mL	0.49 ng/mL	



The Product Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (used for MPCLIA) (Cat. No. MPC-A005) is high stability. The accelerated stability of the product within 10 days at 37°C with no more than 10% performance decrease.



The Product Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (used for MPCLIA) (Cat. No. MPC-A005) is high stability. After freezing and thawing for 3 times, the activity of the product has no more than 10% performance decrease.



The Product Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) (used for MPCLIA) (Cat. No. MPC-A005) is high stability. After reconstitution, the beads can be stored at 2-8°C for 1 month at liquid state, 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 Anti-His Tag-coupled Magnetic Beads (used for MPCLIA) in chemiluminescence immunoassay?
   The Anti-His Tag-coupled Magnetic Beads should be used together with different Acridine ester markers such

as Streptavidin 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 protein, the Acridine ester markers should not cross-react with Anti-His Tag-coupled Magnetic Beads or the His Tagged protein, and the Anti-His Tag-coupled Magnetic Beads should only bind to the His Tagged protein.

2. How long can Anti-His Tag-coupled Magnetic Beads be used in a system reagent bottle after being diluted into a certain concentration?

   After diluting Anti-His Tag-coupled Magnetic Beads to a certain concentration for experiments, it is recommended to use it within one month.