SARS-CoV-2 Spike S1 protein-coupled magnetic beads

Catalog Number：AMS.MBS-K001-2mg Beads
AMS.MBS-K001-10mg Beads

Specifications

Table 1. Beads details

Items	Size	Size
Beads Size	2 mg	10 mg
Particle size	2 μm	2 μm
Beads Surface	hydrophilic	hydrophilic
Coupled amount of S1 protein	50 μg	250 μg
Capacity	> 200 pmol / mg beads > 40 μg anti-SARS2-CoV-2 S1 antibody / mg beads > 20 μg ACE2 protein / mg beads	> 200 pmol / mg beads > 40 μg anti-SARS2-CoV-2 S1 antibody / mg beads > 20 μg ACE2 protein / mg beads
Formulation	Lyophilized from 0.22 μm filtered solution in PBS, 0.05% Tween-20, pH7.4, with 10% Trehalose	Lyophilized from 0.22 μm filtered solution in PBS, 0.05% Tween-20, pH7.4, with 10% Trehalose
Reconstitution	2 mL ultrapure water (1mg beads/mL)	10 mL ultrapure water (1mg beads/mL)

Product description

The magnetic beads are pre-coupled with biotinylated SARS-CoV-2 Spike S1 protein to streptavidin conjugated magnetic beads, because of the extraordinarily high affinity between biotin and streptavidin, the biotinylated SARS-CoV-2 Spike S1 protein can bind to the SA beads irreversibly. The antigen coupled beads can be used for capturing anti-SARS-CoV-2 S1 antibody or ACE2 protein, isolating anti-SARS-CoV-2 antibody displaying yeast cells and antigen-inhibitor binding assays.

IMPORTANT: Please carefully read this manual before performing your experiment.

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Applications

The SARS-CoV-2 Spike S1 protein-coupled magnetic beads can be applied in many assays, including immunocapture of Anti-SARS-CoV-2 S1 antibodies, protein-protein binding assay or antigen-antibody binding assay, antibody screening, bio panning and cell isolation.

Before starting these Assays, all reagents and materials required in the experiment should be prepared

Materials and Reagents Preparation

The required materials and reagents are prepared according to Table 2.

Table 2. Materials and Reagents Preparation

Name	Specifications	Details	Remark
S1 protein pre-coupled magnetic beads	2 mg Beads	25 μg S1 protein / mg Beads	Reconstitute the 2mg Beads with 2 mL ultrapure water (1mg beads/mL)
	10 mg Beads (5 mg*2)	25 μg S1 protein / mg Beads	Reconstitute 5mg/bottle Beads with 5 mL ultrapure water (1mg 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.	When the sample volume more than 0.2 mL, we need this type Magnetic separator, it is often used in immunocapturing, Bio panning or cell isolation.
	For 96 well plate		When the sample volume less than 0.2 mL, for example, when do binding Assay, we can use the 96 well plate separator.
Mixing device with tilting and rotation	According to your experimental		During the reaction, you should use a suitable mixer and do not mix the sample vigorously.
Assay / Washing Buffer	PBS, pH 7.3	No BSA and Tween20	The PBS Buffer is often used for the pretreatment of mass spectrometry (MS) samples.
	PBS, pH 7.3, 0.05% Tween-20	No BSA	If your sample could be disturbed by BSA, you can use the no BSA Buffer. For many applications, adding a detergent, such as 0.01–0.1% Tween™ 20 to the Assay/washing buffers could reduce non-specific binding.
	PBS, pH 7.3, 0.05% BSA, 0.05% Tween-20	BSA and Tween20	The Buffer often used in the no serum sample's Binding Assay.
	Special Assay / Blocking Buffer (Cat. No. TAS001-C05)	No BSA, it is a protein-Based Blocking Buffer	The Buffer can be use in binding Assay of serum sample, but if you need capture sample to do MS, you need to do some follow-up.
Pipette	According to your		

	experimental		
Tubes or 96 well plate	According to your experimental		If no BSA protectant is added to your reaction system, please select low adsorption tubes. If you need a 96 well plate, please select the appropriate plate and the corresponding Magnetic separator stand.
Some other Materials and Reagents	According to your experimental		For example, magnetic separation column (phage display), and Regeneration reagents (MS)

General guidelines

1. Because the particle size of magnetic beads is 2 μm, it may be easy to stick to the side of the bottle in the shipping process, before open the lip, please place the bottle at the right direction, and tap the bottle to ensure the beads to the bottom of the bottle.
2. Use a suitable magnetic separator, and keep the tubes or plates on the magnetic separator for 1-2 minutes to ensure that all the beads are collected on the tube wall and separation from the supernatant.
3. While removing the supernatant from beads, use caution not to lose the beads.
4. Use a Mixing device to tilt/rotate the tubes so the beads do not settle at the tube bottom, and make sure the reaction between samples is adequate.
5. For small volume reactions, select the appropriate size of the tubes to reduce sample loss.
6. Avoid air bubbles during pipetting.
7. The Assay/Washing Buffer will directly affect the results, it's important to choose the right Buffer.
8. Before the formal experiment, an optimization or a pilot test is highly suggested.

General Protocols

magnetic beads Reconstitution

To make sure the beads are all sucked out, you can transfer all the beads in two times, for example, when dealing with two milligrams of magnetic beads, you can first add 1 mL ultrapure water to the beads and transfer them to your tube, and then add another 1mL ultrapure water to the remaining magnetic beads, and transfer them all to your experimental tubes.

Wash the magnetic beads

Before the reaction, it is necessary to wash the magnetic beads and remove the trehalose from the formulation buffer, and change the buffer to your Assay / Washing Buffer.

1. Place the tube with Reconstitute 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 equal volume (or at least 1000μL) of Assay/ Washing Buffer by a 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.

Resuspend the Beads to a suitable volume

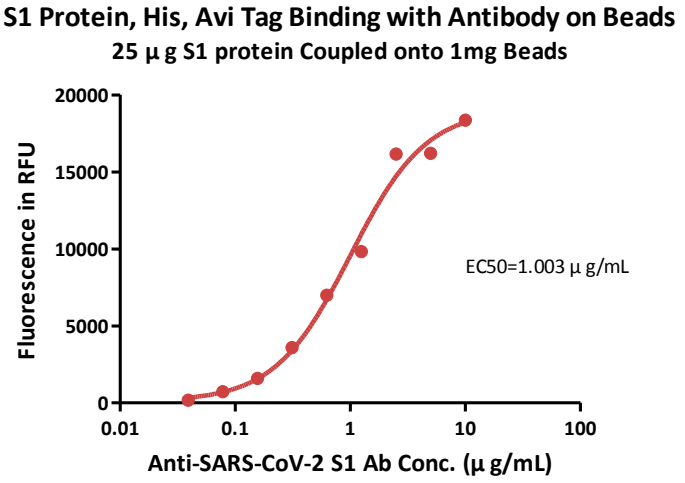
Because of experimental differences, the final resuspension volume of the beads is also different in using process, but usually within a reasonable range (0.1-1mL / mg Beads), the Resuspend buffer is Assay/washing Buffer.

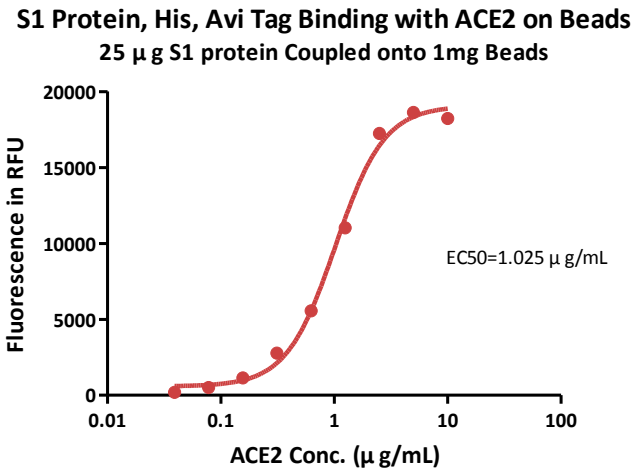
Application protocols

Binding Assay

1. Reconstitute and wash the magnetic beads according to "General Protocols" before use.
2. Add 1mL Assay/Washing Buffer per mg Beads to resuspend the Beads. The Assay/Washing Buffer is often use PBS, pH 7.3, with 0.05% BSA and 0.05% Tween-20, if your sample could be disturbed by BSA, you can use the no BSA Buffer. When you detect serum sample, please chose a Special Assay / Blocking Buffer (Cat. No. TAS001-C05) to help reducing the background signal.
3. Sample Dilution: Dilute the Anti-SARS2-CoV-2 S1 antibodies from 10μg/mL to 0.039μg/mL by Assay buffer.
4.Add 100μL Beads to each tubes or plate well, place the beads on the magnetic separator for 2 min, remove the supernatant.
5.Add the diluted sample by 100μL/well or tube to the pelleted beads, and mix the beads with samples by mixer, add 100 μL of assay buffer, as a blank control, into "BLK" wells containing the beads.
6. Cover the tubes on a Rotator or place the plate on a plate mixer, and incubate for 60 minutes at room temperature.
7. Place the tube/plate on the magnetic separator for 2 min, remove the supernatant.
8. Remove the tube/plate from the magnetic separator and resuspend the pelleted beads in 200 μL of Assay/ Washing Buffer by a vortex.
9. Wash the beads for a total of 4 times by repeat steps 7–8 three.
10.After the last wash, we need to remove the supernatant in the same way as step7.
11.Dilute your Secondary Antibody by Assay buffer, and add 100μL the secondary antibody (at an appropriate dilution ratio) to the beads. You can use an PE anti-Human IgG Fc (Biolegend, Cat. No. 409304) at 1:200 to detect your human IgG antibody samples, or other fluorescent labeled secondary antibodies.
12. Cover the tubes on a rotator or place the plate on a plate mixer, and incubate for 60 minutes at room temperature.
13. Repeat steps 7-8 for a total of four washes with Assay/Washing buffer.
14. After the last wash, we need remove the supernatant in the same way as step7.
15. Add 100 μL of Assay/ Washing Buffer to the beads, and resuspend the Beads.
16. Remove the 100μL beads into a Corning 96-well black plate, make the beads at suspend state.
17. Read the plate at excitation 488 nm / emission 575m on a plate reader within 10 min (Avoid the precipitation of the beads).

Example Data





Immunocapturing of Anti-SARS2-CoV-2 S1 antibodies

1. Refer to “General Protocols” for reconstitution and washing steps before use.
2. Sample preparation: If you need dilution, please dilute with Assay/Washing Buffer, it is usually better to keep the antibody concentration at 50-100 μg/mL, if the antibody concentration is not high enough, you can repeat add samples to beads several times, each time, add 1mL sample to 1mg beads, at the second time, add 1mL sample to the antibody-bonded beads, you can usually repeat with 3-4 times.

The Assay Buffer is often No BSA Buffer, such as PBS, pH 7.3, or add 0.05% Tween-20 detergent, when you need capturing antibodies from serum sample, please dilute the sample at 5~20% serum conc. with Special Assay / Blocking Buffer (Cat. No. TAS001-C05) .

3. Add your antibody sample to the Beads in tubes, cover the tubes on a Rotator, and incubate for 60 minutes at room temperature while rotating.
4. Place the tube on the magnetic separator for 2 min, remove the sample supernatant to a tube, remain the sample supernatant, don't throw away. (In case that samples are no bonded to the beads completely, you confirm that by measuring the conc. of remain antibody in sample supernatant).
5. Remove the tube from the magnetic separator and resuspend the pelleted beads in Washing Buffer (PBS, pH 7.3, 1mL/mg Beads) by a vortex.
6. Place the tube on the magnetic separator for 2 min, remove the wash supernatant.
7. Remove the tube from the magnetic separator and resuspend the pelleted beads in Washing Buffer (PBS, pH 7.3, 1mL/mg Beads) by a vortex.
8. Wash the beads for a total of 4 times by repeat steps 5–6 three.
9. After the last wash, we need remove the supernatant according the step6.
10. Antibody dissociation: add 50μL/mg beads of Regeneration Buffer to the sample bonded beads, and incubate for 5 minutes at room temperature. For example, when we use 5mg beads, we should add 250μL Regeneration Buffer into the beads.
11. Place the beads with Regeneration buffer in tube on the magnetic separator for 2 min, remove the dissociation supernatant to a tube, and remain the dissociation supernatant, don't throw away.
12. The Regeneration Buffer is often 3 M MgCl2, or 10mM Glycine-HCl, pH2.5.
13. If you choose the 3 M MgCl2 as the regeneration buffer, you just need 1 time regeneration, but the dissociation supernatant needs to be further desalted and concentrated. (see the step14)

When you use 10mM Glycine-HCl, pH2.5 as regeneration buffer, we need to make sure that your antibody is stable in glycine, and you need to repeat regeneration steps twice in the same way of step11.

At last, add 2M tris to neutralize the system at the ratio of 1:40 (1μL 2M tris, into 40μL 10mM Glycine-HCl, pH2.5 system).

14. Desalting and Concentration of dissociation supernatant

1) Before the dissociation antibody sample to MS detection, we need to change the sample buffer to PBS, pH 7.3. Usually the dissociation sample volume is small, when the volume is small than 200μL, it is highly recommend to add some PBS buffer, pH 7.3 to ensure the volume is feasible for the desalting process.

2) Use a desalting column to remove the MgCl2 from sample. (i.e. Thermo Fisher Zeba Spin Desalting Columns, 7K MWCO, Cat. No. Pierce-89890).

3) Measure the concentrating of the desalted sample using UV-Vis method.

4) If the concentration of the desalted sample is lower than 0.5 mg/mL, you can concentrate the sample using an ultrafiltration concentrator, and make sure the concentration is above 0.5 mg/mL.

15. Detect the final sample.

You can detect your sample by MS or any other techniques to sequence the antibodies.

Yeast cell isolation.

1. Refer to “General Protocols” for reconstitution and washing steps before use.
2. Yeast preparation: Take $1X10^{10}$ induced expression yeast cells to a sterile tube, add 20 mL PBS (with 1% FBS) to wash the yeast cells twice, then add 5 mL PBS (with 1% FBS) for resuspension, Keep the yeast at 4C° on ice.
3. Add the 5mL yeast to 2mg beads, incubate for 10 minutes at 4C°, flipping the yeast-beads mixture every 2 minutes to avoid precipitation.
4. Add 40 mL PBS (with 1% FBS), and centrifuge the yeast-Beads at 3000 rpm/min for 3-5 minutes, remove the supernatant.
5. Resuspend the yeast with 50 mL PBS (with 1% FBS) by gently shaking, then filter with 200 purpose sterile nylon mesh into a 50 mL sterile centrifugal tube.
6. Place a magnetic separator Column on the magnetic separator, wash with 3mL pre-cooled PBS (with 1% FBS) buffer.
7. Add 7mL yeast-Beads suspension to the column, after the suspension elutes out, the yeast-beads are attached to the magnetic separator, remove the column from the magnetic separator and put it back immediately (Prevent local aggregation).
8. Add 1mL PBS (with 1% FBS) to wash the column.
9. Repeat the step7-8, make the whole 50 mL yeast-Beads through the column.
10. Add 3mL PBS (with 1% FBS) to wash the column for 3 times.
11. Remove the column from the magnetic separator, add 7mL PBS (with 1% FBS), use a syringe piston to push the yeast solution into a 15 mL sterile centrifugal tube.
12. Expanding the first screened yeast cell culture.
13. The second screening of yeast by magnetic beads, the main procedure is similar to the first screening, but usually the second screening beads are different from the first. It often use an anti-Biotin Beads, and need add the biotinylated antigen (S1 protein), to avoid screening out some unwanted clones of anti-secondary Ab.
14. Flow cytometry (the third screening)
    1) Add a primary antibody (i.e. anti -cmyc, 1:200) to 500μL screened yeast, and add 100 nM Biotinylated S1 protein, incubate for 30 minutes at 4C°.
    2) Add 500μL PBS (with 1% FBS) to wash the column for 2 times.
    3) Add 500μL 1:200 FITC-Secondary antibody and APC-Streptavidin, incubate for 30 minutes at 4C°.
    4) Add 500μL PBS (with 1% FBS) to wash the column for 2 times.
    5) Resuspend the yeast with 4 mL PBS (with 1% FBS), Keep it on the ice before analysis in flow cytometry.
    6) Screen out the primary and antigen- positive bind yeast, and do the expanding.
15. The last screening: The main procedure is same as the third screening. In order to avoid screening out some scFv clones which can bind APC-Streptavidin you can use a PE-Streptavidin or PE-Neutravidin instead of the APC-Streptavidin.

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