Color-dyed/Fluorescent Microspheres

For Lateral Flow

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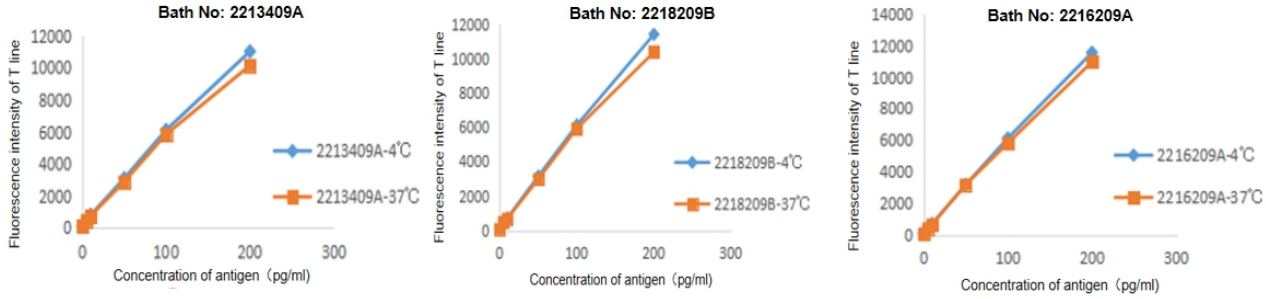
Part 1. Basical Questions

Q: What are the requirements for the storage conditions of microspheres?
A: Carboxyl-coated microspheres: 2-25℃, for long-term storage, 2-8℃ is recommended. Streptavidin-coated microspheres: 2-8℃.
Note: 1. Do not freeze.
2. Protect fluorescent microspheres from light.
3. It is not recommended to store it close to the inner wall of the refrigerator when stored at 2-8°C. Also, the bottle should be avoided to be inverted.

Q: What test items are microspheres immunochromatography applied to?
A: The applicable test items mainly include:
Tumor marker detection: pepsinogen 1 (PG1), pepsinogen 2 (PG2), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), etc. Inflammatory indicators such as interleukin-6 (IL-6), procalcitonin (PCT), etc.
Respiratory infectious disease detection: SARS-CoV antigen detection, A/B virus antibody detection, etc.
Food safety detection: aflatoxin B1 and ochratoxin A, etc.
Aquatic product safety testing: chloramphenicol, etc.

Q: How stable is the test strip prepared by VDO microspheres?
A: As an example, 2019-nCoV antigen detection strips prepared from VDO Biotech's three batches of time-resolved fluorescent microspheres showed stable fluorescence intensity of T-line after accelerating at 37℃ for a week, indicating that the test strips prepared by VDO microspheres have good stability.



Q: What are the differences between time-resolved fluorescent microspheres and ordinary fluorescent microspheres?
A: Time-resolved fluorescent microspheres containing rare earth elements have a long fluorescence half-life, which can reduce background signal and improve the detection sensitivity. Therefore, when measuring, just delaying the measurement time until the fluorescence of background material fully attenuated can effectively eliminate interference of other non-specific fluorescence. Moreover, the long Stokes shift can eliminate the matrix interference and improve the detection accuracy. In summary, time-resolved fluorescent microspheres have great advantages over ordinary fluorescent microspheres.

Q: What is the production capacity of VDO microspheres, is it stable, and how long is the delivery time?

A: Production capacity is stable, as follows:

Production capacity

Time-resolved fluorescent microspheres: Single batch capacity is up to 5L.

Color-dyed microspheres: Single batch capacity is up to 20L.

Delivery time

Usually 2 weeks.

Q: How about the stability of different batches of microspheres?

A: The inter-batch difference of test strips prepared by our microspheres is generally controlled within 15%. Take time-resolved fluorescent microspheres (300nm) as an example:

Table 1: The mean value of T-line fluorescence intensity of three batches of products

n-CoV N蛋白 (pg/mL)	2213409A	2216209A	2218209B	CV
0	83	78	96	10.85%
5	470	494	504	3.57%
10	791	701	724	6.33%
50	3086	3182	3155	1.58%
100	6123	6135	6131	0.10%
200	11059	11567	11424	2.31%

As shown in Table1, the CV is less than 15%.

Q: Which methodology is the most commonly used for immunochromatography? Are there other methodologies?

A: The most commonly used is the double antibody sandwich assay for antigen detection, such as SARS-CoV-2 antigen detection, detection of tumor markers. There are some items using the double antigen sandwich assay for antibody detection, such as Helicobacter pylori project (HP). And there is also a competition assay, which can be used for small molecules detection, such as vitamin D (VD).

Q: How to choose the particle size of microspheres?

A: In general, the smaller the size of the microspheres the more complete the release, which is more conducive to a full reaction and ultimately helps to improve the linearity of the results. The larger the size of the microspheres the higher the detection value, which is more advantageous in improving the detection sensitivity. Customers can choose the appropriate particle size of microspheres according to the specific performance needs of the project.

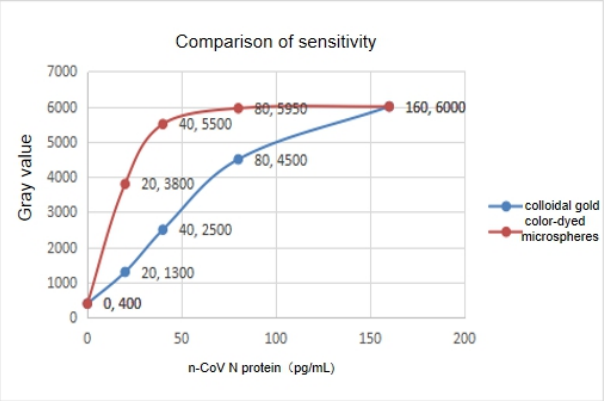
Q: What are the advantages of VDO color-dyed microspheres compared with competitive products?

A: High and stable production capacity, with a single batch production capacity of up to 20L; excellent product performance, better uniformity and stability; high sensitivity and

cost-effective.

Q: For color-dyed/fluorescent microspheres products, what else technical support or service can VDO Biotech provide?
A: We can provide one-stop service from raw materials, consumables to professional technical support, specifically including microsphere trial, coupling process, test strip preparation process, glass fiber membrane, NC membrane and other consumables, as well as assisting customers in process optimization, antibody or antigen labeling.
About glass fiber membrane, we have screened a glass fiber membrane (Cat# VHC06001, VHC06002), which can be used as conjugate pad without pretreatment. It has good release effect saving labor and time cost for customers.

Q: What are the advantages of color-dyed microspheres compared to colloidal gold?
A: Sensitivity The large particle size of color-dyed microspheres corresponds to a higher sensitivity than colloidal gold. As shown in the figure below:



Product color Colloidal gold has a single color, and its application is limited; while color-dyed microspheres have multiple colors and can be used for multiplex detection.
Labeling method Colloidal gold binds with antibodies or antigens through physical adsorption, and the binding is unstable; while color microspheres bind with antibodies or antigens through covalent bonds, and the binding is relatively stable.
Product capacity In the case of SARS-CoV-2 antigen detection reagent, for example, each liter of colloidal gold can produce 10-20,000 detection products, while each liter of color-dyed microspheres can produce about 20 million detection products.

Q: Are antibody pairs suitable for colloidal gold necessarily suitable for color-dyed microspheres?
A: Not necessarily. The difference in labeling principle between colloidal gold and color-dyed microspheres makes the requirements for antibodies different.Colloidal gold binds with antibodies through physical adsorption, while color-dyed microspheres bind with antibodies through covalent bonds. The performance requirements (e.g., purity) of antibodies between these two approaches will be different.

Q: Take SARS-CoV-2 antigen as an example, how many test reagents can be produced

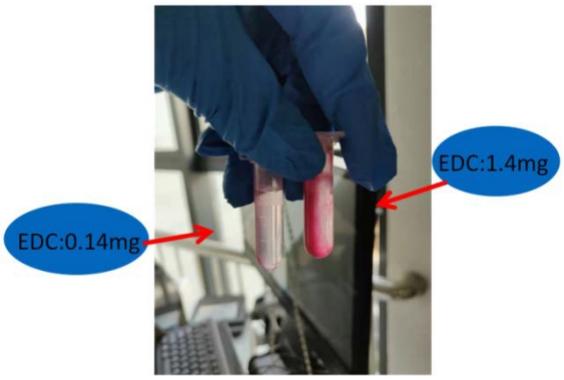
by 1L of color-dyed microspheres?

A: Each test requires 1.8μg of color-dyed microspheres (400nm particle size), so 25mL/bottle of color-dyed microspheres (4% solid content) would produce 555,555 tests. By analogy, 1L of color-dyed microspheres can produce about 20 million tests.

Part 2. Questions of Coupling

Q: What should be done to adjust for severe wall adherence when centrifuging the activated microspheres?

A: Reduce the amount of activator appropriately. As shown in the figure below, when the EDC reached 1.4 mg (excessive amount), the microspheres aggregated after centrifugation and had poor morphology, which seriously affected the labeling.



Q: How to solve the problem of severe wall adhesion phenomenon during centrifugal cleaning after the coupling and blocking of microspheres-antibodies? (Activation and coupling processes are normal)

A: If activation, coupling and blocking processes are normal, and the wall adhesion occurs only during the final centrifugal cleaning. It is recommended to optimize the conditions of cleaning buffer, such as: ion concentration, pH and the amount of surfactant.

Q: Aggregation occurs during antibody conjugation. How to adjust the process?

A: The following methods can be tried successively:

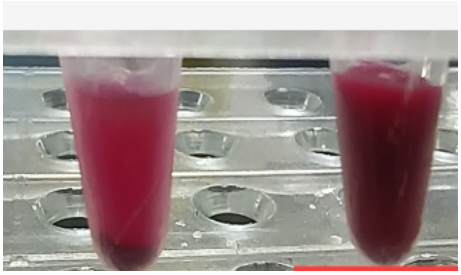
1. Dilute the antibody before adding. For example, for a 1 mL reaction volume, the reaction volume can be added to 0.75mL firstly, then dilute the antibody to 0.25mL and finally mix and couple;
2. Optimize the ion concentration of coupling buffer, which can be reduced or increased appropriately;
3. Optimize the pH of coupling buffer, which can be reduced or increased appropriately;
4. Change the type of coupling buffer, which generally includes MES, PBS, HEPES, boric acid buffer, etc.
5. If the above methods still cannot solve the problem, it is recommended to replace the antibody and re-screen the antibody.

Q: By reducing the ion concentration of coupling buffer, aggregation phenomenon has been greatly improved, and sensitivity also meets the requirements. But the T line is very thin, and the band is not wide enough, how to solve it? (The coupling mass ratio of

microspheres to antibody is 10:1)

A: Reduce the amount of antibody appropriately, such as adjusting coupling mass ratio of microspheres to antibody to 20:1 or 30:1.

Q: What is the effect of precipitation during storage after microspheres-antibody coupled and how to solve it?



A: The precipitation may affect release of the test strip, which may lead to false positive results.

The following points can be tried successively to adjust the process:

1. Appropriately increase the pH of the preservation solution, for example, pH 7.5, 8.0, 8.5 and other conditions.
2. Appropriately reduce or increase the ion concentration of the preservation solution.
3. Optimize the coupling process.
4. Re-screen antibodies.

Q: Which ultrasound tool (probe type/ultrasound machine) is better for batch labeling, e.g., 100mL, 200mL?

A: The concentration of microspheres in the reaction system is recommended to be 1 or 2 mg/mL. If reaction volume is 100 to 200mL, it is recommended to adopt probe type ultrasound with a power of 600 to 800W. The specific operation is to place in an ice-water bath, ultrasonic for 3s, pause for 5s, and ultrasonic time is 3-5 min.

Q: When using BSA blocking buffer,will blocking effect be better by increasing the concentration of BSA?

A: Properly increasing the BSA content and blocking time can improve blocking effect.

Part 3. Questions of Microspheres Release

Q: How to judge the release effect of microspheres?

A: During releasing, there are phenomena such as blocking of microspheres on the conjugate pad, accumulation of microspheres when they reach NC membrane, and (when red latex microspheres are used) the surface of NC membrane is reddish, indicating that the release is poor.

Q: How to solve microspheres release problem?

A: During releasing, there are some phenomena such as blocking of microspheres on the conjugate pad, accumulation of microspheres when they reach NC membrane, and (when

red latex microspheres are used) the surface of NC membrane is reddish, indicating that the overall process of the test strip needs to be optimized. To a large extent, the release of test strip directly reflects the suitability of the labeled antibody in the system. The system includes raw materials, labeling process, sample pad and conjugate pad processing, sample dilut process, etc.

1. **Raw materials** Screen antibodies in advance, some antibodies are not suitable for lateral flow assay.

2. **Labeling process** Normal centrifugation, no agglutination in the coupling and blocking process, and no serious precipitation after storage are important representation of normal labeling process. These factors will directly affect the release of the test strip. Therefore, if one of the steps is abnormal, it is recommended to carry out optimization of pH, ion concentration in the labeling process system, and replacement of the coupling buffer system if necessary.

3. **Sample pad and conjugate pad** At present, many glass fiber membranes have poor hydrophilicity. In order to facilitate microspheres release, the sample pad and conjugate pad are usually pretreated to achieve the purpose of hydrophilicity. Take the sample pad treatment as an example, the recommended treatment buffer is phosphate system containing appropriate amount of casein and surfactant. Casein can reduce the occurrence of non-specific phenomenon, and surfactant can appropriately contribute to microspheres release. For the conjugate pad, we recommend a glass fiber membrane without pretreatment, which has good hydrophilicity and use effects.

4.**Sample diluent** 20mM phosphate buffer, pH 7.4-7.8, containing an appropriate amount of surfactant is recommended.

Q: What should be done when there is difficulty in releasing a saliva sample when testing with color-dyed microspheres test strips?

A: Saliva contains a certain amount of salivary mucin, which will cause microspheres to stick and agglomerate, thus seriously affecting the release. It is recommended to dilute the saliva sample about 5 times or install a filter device in the throat swab tube.

Q: What are the effects of surfactants on microspheres?

A: Generally, only an appropriate amount of surfactant is added to cleaning buffer and preservation solution after blocking. A small amount of surfactant helps to the stability and release of microspheres, but excessive amount may affect the performance of microspheres.

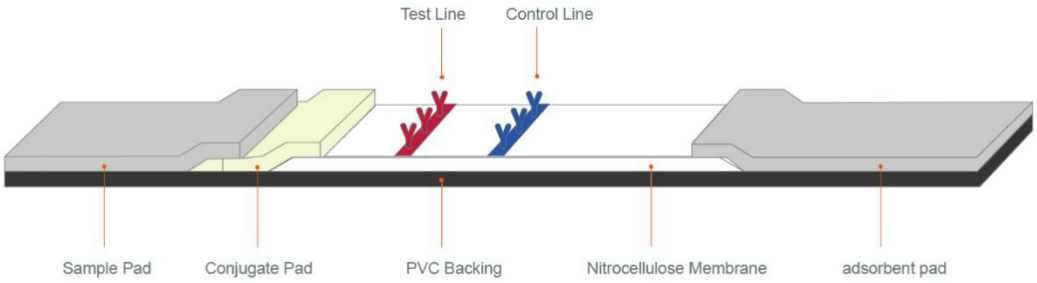
Q: When testing whole blood sample, the release effect of color-dyed microspheres on the test strip is poor, how to deal with it?

A: It is recommended to dilute whole blood samples, generally 3 to 5-fold.

Q: Are glass fiber membrane and NC membrane the same? What is the role of glass fiber membrane?

A: No, glass fiber membrane is made of glass fiber, which is used to carry samples and release microspheres. NC membrane (nitrocellulose membrane) is made of nitrocellulose,

which is used for carrying solid-phase coating of antibody protein and antigen-antibody immune reaction.



Part 4. Questions of Sensitivity

Q: How to improve the reactivity of T-line?

A: When release process is normal, the factors related to the reactivity of T-line include coupling ratio, coating concentration of T-line, and the amount of microspheres on the conjugate pad.

Coupling ratio The binding efficiency of antibody and microspheres is related to antibody itself, coupling buffer system and binding ratio. Take the same project as an example, the antibody activity of different strains or manufacturers is different,as well as binding ability. So, the antibody should be screened firstly.In addition, the coupling solution and buffer also need to be optimized. For example, ion concentration and pH should be optimized to the best level. Finally, optimize the binding ratio between antibody and microspheres.

Coating concentration of T- line Conduct a gradient test on coating concentration, and select the best concentration condition without affecting the background.

Amount of microspheres on conjugate pad Conduct a gradient test on the amount of microspheres used on the conjugate pad, and select the optimal dosage condition without affecting background and release.

Part 5. Questions of Specificity

Q: How to solve the problem of non-specific binding?

A: The factors affecting non-specific binding include antibody itself, labeling process, coating concentration and so on.

1. Due to its own characteristics, some antibodies still show strong non-specific binding even after blocking. Therefore, these antibodies should be abandoned.
2. If weak non-specific binding occurs, the labeling process can be optimized as necessary. For example, appropriately reduce the amount of activator, optimize coupling buffer system and optimize blocking condition (such as appropriately increase protein content of blocking buffer or prolong blocking time).
3. For weak non-specific binding, sometimes proper reduction of coating concentration can also have a good effect.