User's Manual and Instructions

xMag-Streptavidin Microparticles

Catalog Number: Z5060009

Description

xMag-Streptavidin Microparticles are uniform, superparamagnetic composite particles coated with streptavidin. xMag is a new type of super-paramagnetic particle in which the surface of the core Fe_3O_4-SH particle is assembled with a layer of 15 nm colloid gold particles. Streptavidin is a protein (MW of approx. 66 kD) composed of four identical subunits, each containing a high affinity binding site for biotin (Kd = 10^{-15} M). The extremely high affinity and rapid binding between streptavidin and biotin makes xMag-streptavidin microparticles suitable for immunoassay, nucleic acid hybridization and other biological assays.

Feature

- Diameter: 5 μm
- Concentration: 2 mg/ml
- 1 mg xMag can bind at least 100 μg streptavidin through hydrophobic interaction and electrostatic interaction. 1 mg xMag-streptavidin microparticles is sufficient to bind at least 700 pmole free biotin and 200 pmole biotinylated oligonucleotides (10~30 bp).

Application

- By immobilizing biotinylated antibody or nucleic acid onto the surface of xMag-streptavidin microparticles, this product can be used for
- Immunoassays
- Nucleic acid hybridization

Storage

Store at 2 8°C

Shake vigorously by hand or gently pipet up and down to obtain a homogeneous suspension before use. Avoid freezing, heating, vortexing, and centrifugation.

Note: Can not be used in PBST buffer.

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Protocol

Principle

Streptavidin-coupled xMag microparticles are designed as a solid phase matrix for simple and efficient binding for biotinylated compounds such as small molecules, proteins, oligonucleotides, DNA/RNA etc. By using magnetic separators, the streptavidin-coupled xMag microparticles allow isolation and subsequent handling of target molecules in a highly specific manner. Capturing, washing and detection of target molecules can beeasily performed and optimized.

Additional materials and equipment required

- Biotinylated antibody or nucleotides for immobilizing
- Binding&washing buffer: 0.01 M Tris-HCl, pH 7.4, containing 1 mM EDTA and 1 M NaCl
- Blocking buffer (0.01 M Tris-HCl, pH 7.4, containing 5 % skim milk)
- Magnetic separator: a magnetic separator of more than 3 000 Gauss magnetic intensity is needed
- Eppendorf tubes
- Pipette
- Incubator shaker

The following coupling protocol is a suggested procedure and it can be modified substantially with regard to scale and reagent ratios.

Pretreatment of xMag-Streptavidin Microparticles

The xMag-Streptavidin Microparticles should be washed before use in order to remove the NaN_3 which was added as a preservative. The following washing procedure is facilitated by using a magnetic separator:

1. Resuspend the xMag-streptavidin microparticles by gently shaking to obtain a homogeneous suspension.
2. Transfer an appropriate amount of particles to an eppendorf tube, then place the tube on the magnetic separator for 2~3 min.
3. Remove the supernatant using a pipette while keeping the tube on the magnetic separator. Avoid touching the inside wall of the tube.
4. Remove the tube from the magnetic separator. Add the binding&washing buffer along the inside wall of the tube where the particles are collected .Use the same volume as in step 2 above and resuspend the microparticles gently.
5. Repeat steps 3 and 4 twice. The microparticles are now ready to use.

Note: xMag-Streptavidin Microparticles are NOT supplied in RNase-free solution, the following treatment is recommended if the microparticles are to be used in RNA manipulations.

1. Transfer an appropriate amount of pretreated particles to an eppendorf tube, place the tube on the magnetic separator for 2~3 min.
2. Remove the supernatant using a pipette while keeping the tube on the magnetic separator.
3. Remove the tube from the magnetic separator and resuspend the microparticles gently in sterile water containing 0.1% DEPC. Use the same volume as in step 1.
4. Incubate at room temperature for 10~20 minutes. The microparticles are now ready to use.

Immobilization procedures

Procedure for immobilizing biotinylated nucleotides

1. Dissolve the biotinylated DNA/RNA in binding washing buffer to obtain an appropriate concentration for application.
2. Resuspend the xMag-streptavidin microparticles in binding&washing buffer to a final concentration of 2 mg/ml, or to a concentration suitable for the application of choice.
3. Remove the supernatant using a pipette while keep the tube on the magnetic separator. Then add an appropriate amount of biotinylated DNA or biotinylated RNA to the xMag-streptavidin microparticles.

4. Incubate the mixture in the incubator shaker for 5~10 min at 37 °C and 120 rpm or occasionally mix it by gently tapping the tubes. The optimal incubation time depends on the size of DNA/RNA fragments, longer DNA/RNA fragments may need longer incubation time.
5. Separate the particles, which now are coated with the biotinylated DNA/RNA fragment using a magnetic separator. Leave the tube on the magnetic separator for 2-3min.
6. Wash the particles 2-3 times with binding&washing buffer, using a magnetic separator as describe above.
7. If necessary, add the blocking reagent to the particles, then shake the tube at 37°C, 170 rpm for 30 min~2 hr to block the particles. You may select other blocking reagents and adjust the blocking condition according to your own experiment.
8. Resuspend the particles in the appropriate buffer according to your following application.

Procedure for immobilizing biotinylated protein or antibody

xMag-streptavidin microparticles is ideal for the immobilization of biotinylated proteins and immunoglobulins. Below is a suggested protocol for immobilization of biotinylated antibodies:

1. Calculate the amount of biotinylated antibodies needed for the experiment. 1 mg of xMag-streptavidin microparticles is sufficient for immobilizing 25-30 μg antibodies.
2. Incubate at room temperature for 30 minutes with gentle rotation of the tube.
3. Separate the xMag-streptavidin microparticles which now are coated with biotinylated antibodies using a magnetic separator for 2-3 minutes.
4. Wash 2-3 times in PBS (with 0.1% BSA) using a magnetic separator
5. Resuspend to the desired concentration for your specific target capture or downstream application.

Immoblization procedures for other biotinylated ligands

xMag-streptavidin microparticles may also be used for immobilizing other biotinylated ligands, such as biotinylated protein, biotinylated polypeptide. You may optimize your own experiment according to the above procedures for immobilizing biotinylated nucleotides since the fragment size and biotinylation procedures affect the binding capacity of the particles.