cytodiagnostics

PRODUCT DATA SHEET

Zepto™ Mag Carboxyl Microspheres

Description

Cytodiagnostics Zepto™ Mag Carboxyl Microspheres are synthesized using our proprietary process that internally encapsulates paramagnetic particles. With our process, the superparamagnetic microspheres exhibit very high magnetism that ensures fast magnetic concentration in their applications. Carboxyl surface functional groups are available for carbodiimide covalent conjugation of ligands, e.g. proteins, nucleic acids, and molecules with freely accessible primary amines.

Magnetic microspheres are extremely easy to separate by a magnetic field, and are suitable for automation in device and assay design. They are used extensively in diagnostics and other applications including biomolecule purification and concentration, cell capture and collection, and many others. With a simple magnetic concentration, the purification or washing is highly efficient, convenient, and rapid. These microspheres are hydrophilic, so they are easily re-dispersed in buffers upon removal of the magnetic field.

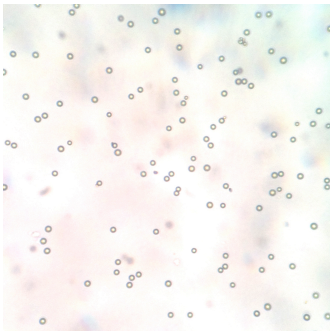

Figure 1. Morphology of Zepto™ Mag Carboxyl Microspheres.

Characteristics

Diameters: 2.5±0.3 μm
Concentration: 2E8/mL in ddH2O
Surface Functional Group: -COOH
Carboxyl Parking Area: ~2.5 nm2/-COOH group

Content

Zepto™ Mag Microspheres: supplied in 5 mL (Cat. #ZBCM-0-5ML) format.

Storage/Stability

This product should be stored at 2-8°C. Avoid freezing, and drying. For the best consistency and stability, the microspheres are best used at a pH from 3 to 8, temperature up to 60 degrees, and salt concentration up to 0.5 M. Product is stable for at least 12 months when stored under the recommended conditions.

Usage Guidelines

Covalent coupling of microspheres via carbodiimide coupling chemistry

Zepto™ Mag Carboxyl Microspheres are designed for surface functionalization with molecules containing primary amines via carbodiimide crosslinkers such as 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). For example, streptavidin-conjugated microspheres for capturing biotin-labeled biomolecules. Different molecules may require different conjugation and detection assay conditions for optimal results. Researchers are advised to optimize parameters such as EDC and ligand concentrations (ratios), for their specific application, see page 3. The following procedure provides general guidelines for conjugation of Protein A to Zepto™ Mag Carboxyl Microspheres, and for their use in downstream assays.

Two-step conjugation procedure

A typical conjugation reaction utilizes 10 million Zepto™ Mag Carboxyl Microspheres. The following reagents are required and not provided with the product:

- Activation buffer: MES buffer (2-(N-morpholino)ethanesulfonic acid) 50 mM, pH 5.5
- Washing buffer: 0.1X PBST (TWEEN20: 0.05% w/v)
- Storage buffer: 1% bovine serum albumin in 0.1X PBST (Sigma, A3059), 0.09% sodium azide
- EDC (Sigma E1769): 1 mg/mL in activation buffer, freshly prepared
- Protein A: 1 mg/mL in 0.5X PBS

919 Fraser Drive Unit 11, Burlington, ON, L7L 4X8, Canada
Tel: 866-344-3954 Fax: 289-288-0122
www.cytodiagnostics.com

1. Vortex the product bottle before use to ensure homogeneous suspension of the microspheres
2. Immediately aliquot 10 million microspheres (100 μL of stock solution as provided)
3. Remove supernatant and add 500 μL of EDC
4. Mix well and activate for 30 min at room temperature
5. Add 500 μL of washing buffer and mix well
6. Place the microsphere tube against a magnet or on a magnetic rack for 1 min
7. Gently remove washing buffer away from the microspheres and add 25 μL of Protein A Solution
8. Mix well and incubate for 4 hours at room temperature with constant rotation
9. Add 500 μL of washing buffer and mix well
10. Place the microsphere tube against a magnet or on a magnetic rack for 1 min
11. Gently remove washing buffer away from the microspheres and add 500 μL of washing buffer
12. Place the microsphere tube against a magnet or on a magnet rack for 1 min
13. Gently remove washing buffer away from the microspheres and add 250 μL of storage buffer and mix well
14. Incubate for 1 hour at room temperature with rotation
15. Conjugated microspheres are now ready for your use in assays. Alternatively, store at 4°C protected from light until use

IgG binding assay by Protein A-conjugated magnetic microspheres

Protein A binds strongly to IgG's of different species. These microspheres are an effective adsorbent to remove Fc fragments during Fab fragment preparation, and in immunoassay systems. In a biological sample, IgG molecules bind to the microspheres, which can then be concentrated and purified.

The following reagents are required and not provided with the product:

Elution buffer: (0.1 M glycine, 0.15M NaCl, pH 2.5)
Neutralization buffer: 1M Tris, pH 8

1. Aliquot 10 μL of conjugated spheres as prepared above
2. Add 100 μL of analyte standard or sample solution and mix well
3. Incubate for 1 hour at room temperature with constant rotation
4. Place the microsphere tube against a magnet or on a magnetic rack for 1 min
5. Remove the solution away from the magnet and keep the solution separately if needed
6. To the microspheres, add 500 μL of elution buffer and incubate for 5 min
7. Place the microsphere tube against a magnet or on a magnetic rack for 1 min
8. Save the clear elute solution away from the microsphere pellet
9. Neutralize the elute solution to ph 7-8 using approximately 25 μL of neutralization buffer

Additional Information

Optimizing the conjugation conditions

The concentration ratio of microspheres to EDC and protein to the activated microspheres ratio may need to be optimized for optimal conjugation.

Sulfo-N-hydroxysuccinimide (NHS) can be added with EDC during activation to increase conjugation efficiency. NHS reacts with the o-acrylisourea intermediate formed by EDC and carboxyl group and replaces it with a more stable amine-reactive ester. The molecular ratio between EDC and NHS is typically at 1:1.

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet available online at www.cytodiagnostics.com for information regarding hazards and safe handling procedures.

Ordering Information

For ordering call 866-344-3954 or visit us online at www.cytodiagnostics.com

919 Fraser Drive Unit 11, Burlington, ON, L7L 4X8, Canada
Tel: 866-344-3954 Fax: 289-288-0122
www.cytodiagnostics.com