cytodiagnostics

PRODUCT DATA SHEET

Zepto™ Ultra Green Carboxyl Microspheres

Description

Cytodiagnostics Zepto™ Ultra Fluorescent Carboxyl Microspheres are internally labeled fluorescent microspheres synthesized using our proprietary technology. Compared to traditional fluorescent microspheres, Cytodiagnostics Zepto™ Ultra Microspheres feature higher quantum yields, sharper emission peaks, and are resistant to photobleaching. Further, our microspheres have increased stability to environmental factors such as high ionic strengths and temperature with an overall longer shelf life.

Emission wavelengths are available at 450nm, 525nm, 575nm, 630nm, and 665nm and are excitable at any wavelength 15nm or below the emission wavelength making them ideal for a wide range of applications, and common emission and excitation filters.

Carboxyl surface functional groups are available for carbodiimide covalent conjugation of ligands, e.g. proteins, nucleic acids, and molecules with accessible primary amines. Such spheres are used extensively in cell tracking, fluidic tracing, biosensing, immunoassays, and imaging applications.

The emission spectrum is illustrated in Figure 1. They are a suitable for a wide range of fluorescent assays such as flow cytometry-based assays. Their Forward Scatter (FSC) vs. Side Scatter (SSC) plot and fluorescence histogram in the FITC channel (405nm, 488nm excitation; 530 nm emission) on a typical flow cytometer are illustrated in Figure 2.

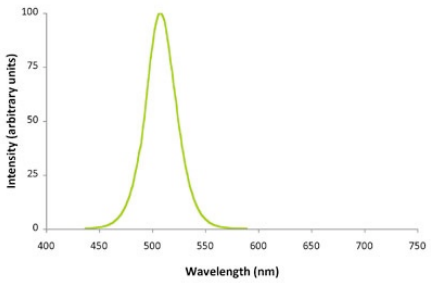

Figure 1. Emission spectrum of Zepto™ Ultra Green Carboxyl Microspheres.

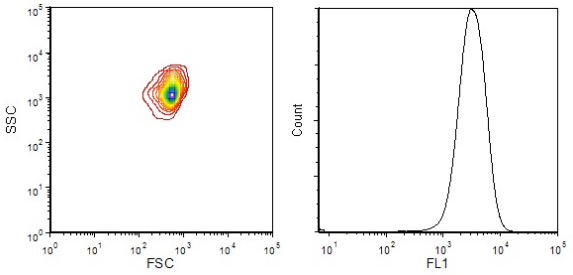

Figure 2. FSC-SSC plot and fluorescence histogram in the FITC channel of Zepto™ Ultra Green Carboxyl Microspheres on a flow cytometer.

Characteristics

Diameters: 2.5±0.3μm
Concentration: 1E+8/mL in ddH2O
Emission max: 525nm
Excitation:<510nm
Surface Functional Group: -COOH
Carboxyl Parking Area: ~2.5 nm2/-COOH group

Content

Zepto™ Ultra Green Carboxyl Microspheres: supplied in 1 mL (Cat. #ZBC-8-1ML) or 5 mL (Cat. #ZBC-8-5ML) formats.

Storage/Stability

This product should be stored at 2-8°C. Avoid freezing, drying or prolonged exposure to light. For the best consistency and stability of microsphere fluorescence, the microspheres are best used in 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™ Ultra Green Carboxyl Microspheres are designed for surface functionalization with molecules containing primary amines via carbodiimide crosslinkers such as 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

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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 antibodies to Zepto™ Ultra Carboxyl Microspheres, and for their use in downstream assays. Multiple antibodies of different specificities, or other molecules of interest, may be conjugated to microspheres emitting different fluorescent colors, allowing for multiplexed detection capabilities in a single assay.

Two-step conjugation procedure

A typical conjugation reaction utilizes 10 million Zepto™ Ultra 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: 0.5X PBS/0.05% (w/v) Tween 20
- Storage buffer: 1% (w/v) bovine serum albumin in 0.1X PBS, 0.05% (w/v) Tween 20, 0.09% (w/v) sodium azide
- EDC (Sigma E1769)/Sulfo-NHS (Sigma 56485) Mix: 5 mg/mL each in activation buffer, freshly prepared
- Protein to be conjugated (e.g. IgG): 0.5 mg/mL in 0.5X PBS

1. Vortex the product bottle before use to ensure homogeneous suspension of the microspheres
2. Immediately aliquot 10 million microspheres (10 µL of stock solution as provided) into a microcentrifuge tube.
3. Add 200 µL of EDC/Sulfo-NHS mixture
4. Mix well and activate for 30 min at room temperature
5. Add 500 µL of washing buffer and mix well
6. Gently pellet at 500 g for 5 min
7. Discard supernatant and add 50 µL of protein to be conjugated
8. Mix well and incubate for 2-4 hours at room temperature with constant rotation
9. Add 500 µL of washing buffer and mix well
10. Gently pellet at 500 g for 5 min
11. Discard supernatant and add 500 µL of washing buffer
12. Gently pellet at 500 g for 5 min
13. Discard supernatant and add 250 µL of storage buffer and mix well
14. Conjugated microspheres are now ready for your use in assays. Alternatively, store at 4°C protected from light until use.

Detection assay

The following procedure is based on a sandwich assay for single analyte detection with flow cytometry. Capture antibodies are conjugated to the microsphere surface using EDC/NHS coupling chemistry as described above. A reporter is typically a fluorophore-labeled antibody that recognizes and binds to a different domain or epitope on the analyte than that of the capture antibody. In the presence of analyte, a sandwich is formed when the microspheres coated with the capture antibody and the reporter antibody bind the analyte. The user may need to optimize assay parameters, or develop direct and multiplex assays for their specific application.

1. Aliquot 10 µL of conjugated spheres as prepared above
2. Add 100 µL of sample solution and mix well
3. Incubate for 1 hour at room temperature with constant rotation
4. Add 500 µL of washing buffer and mix well
5. Gently pellet at 500 g for 5 min
6. Add 5 µL (50 µg/mL) of a fluorophore-labeled reporter antibody in 100 µL 0.5X PBS
7. Incubate for 30 minutes at room temperature with constant rotation
8. Gently pellet at 500 g for 5 min
9. Discard supernatant and add 500 µL of washing buffer and mix well
10. Gently pellet at 500 g for 5 min
11. Resuspend microspheres in washing buffer and analyze by flow cytometry

Flow cytometry analysis

1. Gate on the single population of microspheres on FSC vs. SSC plot (refer to Figure 2)
2. In the fluorescence channel for the reporter, the median values of fluorescence intensity for gated microsphere population determine the “negative” and “positive” by standards. Or the user defines the threshold value for positive detection

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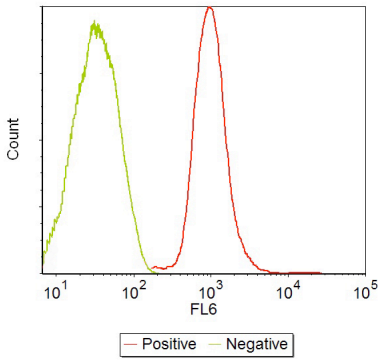

Figure 3. A typical flow cytometry based protein detection assay result using Zepto™ Ultra Carboxyl Microspheres. The figure shows a histogram of a negative sample (green) and a positive sample (red) containing the target of interest. Note the increase in fluorescence of the positive sample due to binding of the fluorophore-labeled reporter antibody.

Additional Information

Optimizing the conjugation conditions

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

Optimization of detection assay conditions

The detection sensitivity is affected by various factors, such as conjugation efficiency, microsphere number and temperature of the detection assay. The user may need to titrate the amount of microspheres for the best sensitivity. For flow cytometry applications, the number of microspheres in gate ranges from 100 to 10000. Fewer microspheres generally leads to better sensitivity but also a higher variance of assays.

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

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