cytodiagnostics

PRODUCT DATA SHEET

Zepto™ Ultra Red 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 APC channel (405nm, 488nm, 633nm excitation; 670nm emission) on a typical flow cytometer are illustrated in Figure 2.

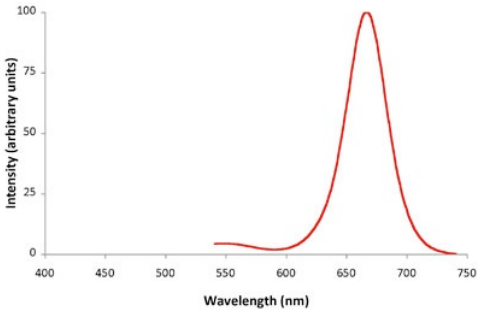

Figure 1. Emission spectrum of Zepto™ Ultra Red Carboxyl Microspheres.

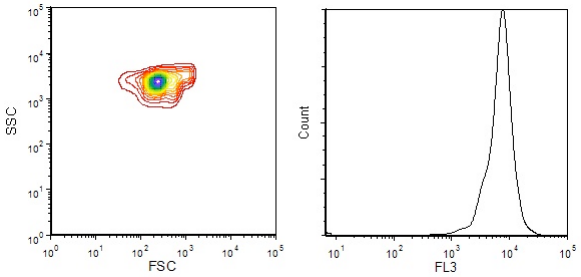

Figure 2. FSC-SSC plot and fluorescence histogram in the APC channel of Zepto™ Mag Ultra Red Carboxyl Microspheres on a flow cytometer.

Characteristics

Diameters: 2.5±0.3μm
Concentration: 1E+8/mL in ddH2O
Emission max: 665nm
Excitation: <650nm
Surface Functional Group: -COOH
Carboxyl Parking Area: ~2.5 nm2/-COOH group

Content

Zepto™ Ultra Red Carboxyl Microspheres: supplied in 1 mL (Cat. #ZBC-10-1ML) or 5 mL (Cat. #ZBC-10-5ML) 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 Red Carboxyl Microspheres are designed for surface functionalization with molecules containing primary amines via carbodiimide crosslinkers such as 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Different

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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 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: 0.1X 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
- IgG: 0.5 mg/mL in 0.5X PBS

1. Vortex the product bottle before using to ensure homogeneous suspension of the microspheres
2. Immediately aliquot 10 million microspheres (10 µL of stock solution as provided)
3. Remove supernatant and add 50 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. Gently pellet at 500 g for 5 min *
7. Discard supernatant and add 50 µL of IgG
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. 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. 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

** The centrifugation speed for pelleting microspheres is a range between 100 to 10,000 g. A lower/more gentle centrifugation speed with longer time is recommended to form a good microsphere pellet, especially after conjugation. Prolonged centrifugation at high speed may aggregate microspheres irreversibly.*

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 via EDC 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 5 µL of reporter antibody (50 µg/mL in TBS, 0.1% BSA)
3. Add 100 µL of analyte standard or sample solution and mix well
4. Incubate for 1 hour at room temperature with constant rotation
5. Add 500 µL of washing buffer and mix well
6. Gently pellet at 500 g for 5 min
7. Discard supernatant, add 500 µL of washing buffer and mix well
8. Gently pellet at 500 g for 5 min
9. 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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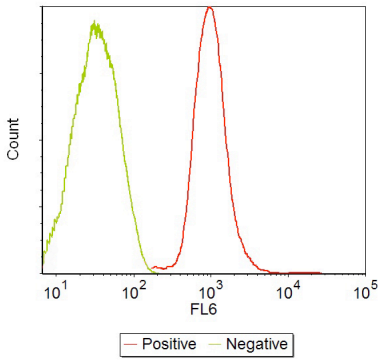

Figure 3. A typical protein detection assay using Zepto™ Ultra Carboxyl Microspheres on flow cytometry. The reporter is Cyto 633 dye detected in FL6 (635 nm excitation; 675 nm emission) of a flow cytometer. Histograms of "negative" (green) and "positive" (red) of the reporter fluorescence are shown above.

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.

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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