

PRODUCT DATA SHEET

Zepto™ Ultra Express Microspheres (2.5µm) 5 Color Kit

Description

Cytodiagnostics Zepto™ Ultra Express Fluorescent Microspheres are internally labeled with fluorescent probes synthesized using a unique proprietary protocol. Compared with solvent swelling or dye entrapment methods, our method produces microspheres with a greater range of fluorescence intensities (over several orders) and a greater number of available emission wavelengths, affording greater flexibility and choice when choosing a fluorescence profile for your assay. In addition, due to the high quantum yield and tunability of our fluorescent probes, these microspheres have much brighter fluorescence intensity and sharper emission peaks with finely tunable emission wavelengths from 450 to 665 nm.

Fluorescent microspheres from other manufacturers require strict excitation conditions that are predisposed by their choices of fluorescent dyes. The fluorescence stability against quenching also varies with different dyes. The Zepto™ Ultra Express Fluorescent Microspheres have greater flexibility in the excitation sources. Therefore, a single excitation source can be used to excite multiple fluorescent colors of our microspheres, making it extremely easy for users to design and conduct labeling experiments and multiplexed bioassays. Our microspheres are resistant to photobleaching and have wide working conditions and their fluorescence is less prone to environmental factors such as salt and heat with a longer shelf life.

Microspheres have wide applications in cell tracking, fluidic tracing, biosensing, immunoassays, and imaging when they are labeled with ligands, e.g. proteins and nucleic acids. The labeling/conjugation methods for traditional microspheres typically rely on carbodiimide coupling reaction, which are challenged with low and inconsistent conjugation efficiencies complicated by crosslinking problem in some cases. The optimal condition of carbodiimide coupling reaction varies with each different molecule. Thus it costs time and efforts of users before they can use these microspheres properly in their applications. Our Zepto™ Ultra Express Microspheres have a significantly simplified, fast and improved conjugation process afforded by their unique metal nanoshell on the surface. The metal nanoshells are specifically designed with high uniformity and affinity to any

molecule with accessible primary amines, thiol, or dithiol groups. You simply mix Zepto™ Ultra Express Microspheres with the molecule(s) of interest, and use, without having to go through the optimization process required with conventional microspheres.

The emission spectra are illustrated in Figure 1. They are a suitable for flow cytometry-based assays. The Forward Scatter (FSC) vs. Side Scatter (SSC) plot and fluorescence histogram of Zepto™ Ultra Express Blue Microspheres in FL4 (405 nm excitation; 455 nm emission) on a typical flow cytometer are illustrated in Figure 2. The plots of the other colors are similar.

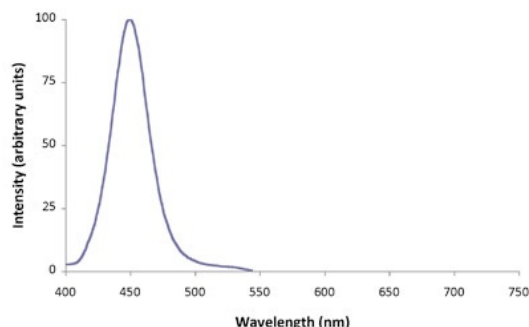


Figure 1. Emission spectra of Zepto™ Ultra Express Microspheres of 5 colors.

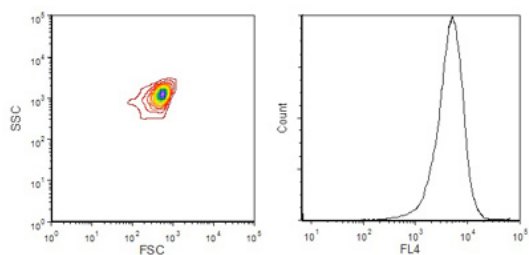


Figure 2. FSC-SSC plot and fluorescence histogram of Zepto™ Ultra Express Blue Microspheres on a flow cytometer.



Characteristics

Diameters: $2.5 \pm 0.3 \mu\text{m}$
Concentration: $1\text{E}8/\text{mL}$ in 2 mM citrate
Emission max: 450, 525, 575 and 665 nm plus non-labeled microspheres
Surface Composition: Metal Nanoshells

Content

Zepto™ Non-Labeled (ex/em): N.A./N.A. (non fluorescent beads)
Zepto™ Ultra Blue (ex/em): <435 nm/450 nm
Zepto™ Ultra Green (ex/em): <510 nm/525 nm
Zepto™ Ultra Orange (ex/em): <560 nm/575 nm
Zepto™ Ultra Red (ex/em): <650 nm/665 nm

Volume: 1 mL each

Storage/Stability

This product should be stored at 2-8°C. For the best consistency and stability of microsphere, the microspheres are best used in pH from 4 to 10 and temperature up to 80 degrees. Product is stable for at least 12 months when stored under the recommended conditions.

Usage Guidelines

Surface Functionalization of Zepto™ Ultra Express Microspheres

Zepto™ Ultra Express Microspheres are designed for fast surface functionalization with molecules containing primary amines, thiol or dithiol groups. The procedure is a simple mix-and-use process. The following protocol provides general guidelines for conjugation of antibodies to Zepto™ Ultra Express 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.

A typical conjugation reaction utilizes 1 million Zepto™ Ultra Express Microspheres. The following reagents are required and not provided with the product:

- Washing buffer: 0.1X PBS containing 0.05% TWEEN20 (PBST)
- Storage buffer: 1% bovine serum albumin (BSA) in PBST (Sigma, A3059)
- IgG: 5 $\mu\text{g}/\text{mL}$ in 0.5X PBS

1. Vortex the product bottle before using to ensure homogeneous suspension of the microspheres
2. Immediately aliquot 1 million microspheres (10 μL of stock solution as provided)
3. Add 10 μL of IgG *
4. Mix well and incubate for 2 hours at room temperature with constant rotation
5. Add 100 μL of washing buffer and mix well
6. Gently pellet at 100 g for 5 min and discard supernatant **
7. Add 100 μL of storage buffer and mix well
8. Incubate for 1 hour at room temperature with rotation
9. Conjugated microspheres are now ready for your use in assays. Alternatively, store at 4°C until use

* The optimal amount of IgG or other molecules may vary with applications. A titration experiment may be needed.

** The centrifugation speed for pelleting microspheres is a range between 100 to 500 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 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 the 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 0.5 μL of conjugated spheres as prepared above (equals about 5,000 microspheres)
2. Add 5 μL of reporter antibody (50 $\mu\text{g}/\text{mL}$ 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 100 g for 5 min
7. Discard supernatant, add 500 μL of washing buffer and mix well

8. Gently pellet at 100 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

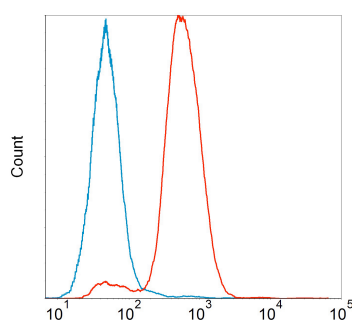


Figure 3. A typical protein detection assay using Zepto™ Ultra Express Microspheres on flow cytometry. The reporter is Cyto dye detected in a flow cytometer. Histograms of “negative” (blue) and “positive” (red) of the reporter fluorescence are shown above.

Additional Information

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.cytodiagnosics.com for information regarding hazards and safe handling procedures.

Ordering Information

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