cytodiagnostics

PRODUCT DATA SHEET

Zepto™ Express Non-Labeled Microspheres

Description

Cytodiagnostics Zepto™ Ultra Express Non-Labeled 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. They are also suitable for flow cytometry-based assays, and are ideal as a non-labeled control when using our fluorescently labeled microspheres.

The labeling/conjugation methods for traditional microspheres typically rely on carbodiimide coupling chemistry, which is challenged with low and inconsistent conjugation efficiencies. Additionally, the optimal conditions for the carbodiimide coupling reaction vary with each different molecule, protein or probe. Thus it costs time, reagent cost, and additional effort before traditional microspheres can properly be used. Our Zepto™ Ultra Express Microspheres have a significantly simplified, fast and improved labeling process imparted by their unique metal nanoshell. 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 them for your application(s), without enduring the optimization process required with conventional microspheres.

The Forward Scatter (FSC) vs. Side Scatter (SSC) plot on a typical flow cytometer is illustrated in Figure 1.

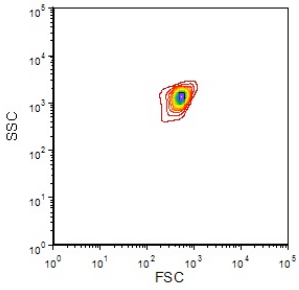

Figure 1. FSC-SSC plot of Zepto™ Ultra Express Non-Labeled Microspheres on a flow cytometer.

Characteristics

Diameters: 2.5±0.3 μm
Concentration: 1E8/mL in 2 mM sodium citrate
Surface Composition: Metal Nanoshells

Content

Zepto™ Ultra Express Non-Labeled Microspheres: supplied in 1 mL (Item #: ZBCE-0-1ML) or 5 mL (Item #: ZBCE-0-5ML) formats.

Storage/Stability

This product should be stored at 2-8°C. For the best consistency and stability of microsphere properties, 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, tiol or dithiol groups. The procedure is simply a 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 μg/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 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 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 microspheres as prepared above (equals about 5,000 microspheres)
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 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 1)
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

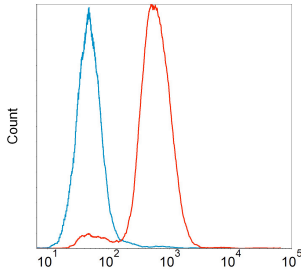

Figure 2. A typical protein detection assay using Zepto™ Ultra Express Microspheres on flow cytometry. The reporter is Cyto dye detected in a flow cytometer. Histograms of “negative” (blue) and “positive” (red) of the reporter fluorescence are shown above.

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.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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