Cell Navigator™ Exosome Fluorescence Staining Kit *Orange Fluorescence*

Catalog number: 22427
Unit size: 100 Tests

Component	Storage	Amount (Cat No. 22427)
Component A: Exsomight™ Orange	Freeze (< -15 °C), Minimize light exposure	1 Vial
Component B: Exsomight™ Quench Buffer	Freeze (< -15 °C), Minimize light exposure	1 Vial (100 µL)
Component C: DMSO	Refrigerated (2-8 °C)	1 Vial (100 µL)

OVERVIEW

The Cell Navigator™ Exosome Fluorescence Staining Kit enables extracellular vesicle (EV) labeling with Exsomight™ Orange, a lipophilic membrane dye designed to improve solubility and reduce aggregation. Conventional dyes like PKH, DiO, and DiI often aggregate, leading to non-specific background and inaccurate EV detection. Exsomight™ Orange addresses these issues by integrating more efficiently into EV membranes, minimizing signal artifacts in fluorescence-based assays.

This kit is optimized for fluorescence nanoparticle tracking analysis (fNTA), flow cytometry, and fluorescence microscopy, enabling high-efficiency EV labeling with minimal background interference. Exsomight™ Orange provides near-complete EV coverage, enhancing the distinction between EVs and non-vesicular particles. Its reduced propensity for aggregation enhances data reliability, particularly in single-vesicle analysis, where dye clusters can confound measurements.

The Cell Navigator™ Exosome Fluorescence Staining Kit supports both quantitative and qualitative EV detection, making it ideal for EV population analysis, cellular uptake studies, and high-resolution imaging. The dye integrates stably into EV membranes, ensuring consistent and reproducible labeling across different sample preparations. By minimizing the common issues of lipophilic dyes, such as aggregation and background fluorescence, this kit provides a more reliable and accurate approach to EV staining, enhancing detection accuracy while minimizing artifacts.

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

Exsomight™ Orange Stock Solution

1. Reconstitute Exsomight™ Orange (Component A) by adding 100 µL of DMSO (Component C) to prepare a 100X stock solution.

   Note: Aliquot any unused Exsomight™ Orange stock solution as single-use aliquots and store at ≤ -20°C. Protect from light and minimize freeze-thaw cycles to maintain reagent stability.

SAMPLE EXPERIMENTAL PROTOCOL

This protocol serves as a general guideline and should be adapted to meet the specific requirements of your experiment.

Extract Exosomes

1. Extract exosomes following your standard protocol.

   Note: Optimal cell density should be determined individually for each cell line.

   Note: For exosome isolation from cell culture medium, the ReadiPrep™ Exosome Isolation Kit (AAT Cat# 60204) is recommended.

Labeling Exosomes

1. Add 100 µL of the exosome solution into a centrifuge tube.
2. Add 1 µL of Exsomight™ Orange stock solution (100X) and incubate at room temperature for 30–60 minutes.

   Note: The optimal incubation time varies based on cell type and exosome concentration. Adjust incubation duration accordingly for each experiment to achieve optimal labeling.

3. Add 1 µL of Exsomight™ Quench Buffer (Component B) to the vial to neutralize any unreacted Exsomight™ Orange dye.
4. Centrifuge the labeled exosomes at 13,000 RPM for 1 hour to pellet them, then carefully resuspend the pellet in 100 µL of PBS.
5. Labeled exosomes can be utilized to investigate endocytosis and other downstream cellular processes.

EXAMPLE DATA ANALYSIS AND FIGURES

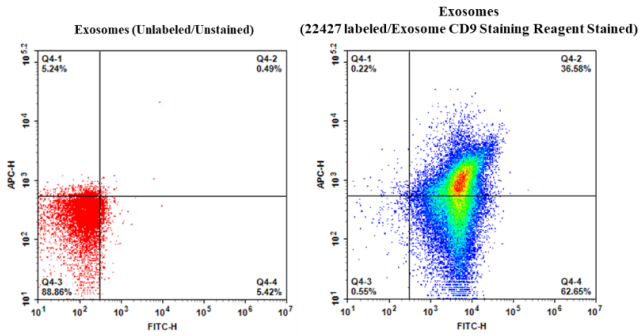

Figure 1. Co-staining of CHO-K1-derived exosomes. Exosomes were isolated from CHO-K1 cells using the ReadiPrep™ Exosome Isolation Kit (AAT Cat# 60204) and subsequently labeled with the Cell Navigator™ Exosome Fluorescence Staining Kit (Cat# 22427). Following this, exosomes were further stained using the Cell Navigator™ Flow Cytometric Exosome Staining Kit (Cat# 22426). Fluorescence signals were acquired using an ACEA NovoCyte flow cytometer, with FITC detection for CD9 staining (Cat# 22426) and APC detection for Exsomight™ Orange labeling (Cat# 22427).

DISCLAIMER

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