SNARF-1-Dextran Conjugate *10,000 MW*

Catalog number: 21267
Unit size: 5 mg

Component	Storage	Amount (Cat No. 21267)
SNARF-1-Dextran Conjugate *10,000 MW*	Freeze (< -15 °C), Minimize light exposure	5 mg

OVERVIEW

SNARF-1-Dextran Conjugate (10,000 MW) consists of the pH-sensitive fluorophore SNARF-1 covalently attached to a 10,000 molecular weight dextran carrier. SNARF-1 exhibits pH-dependent fluorescence emission that shifts with environmental pH, enabling ratiometric measurement of intracellular pH. The dye displays excitation and emission maxima near 549/586 nm under acidic conditions (pH 6) and 576/640 nm under basic conditions (pH 9), allowing quantitative monitoring of intracellular pH changes over a physiologically relevant range. The hydrophilic dextran carrier provides excellent aqueous solubility and serves as a biologically inert tracer for intracellular studies. The 10,000 MW dextran format is well suited for microinjection, cell loading, lineage tracing, neuronal tracing, intracellular transport analysis, and investigations of cell-to-cell communication. SNARF-1-Dextran Conjugate is compatible with fluorescence microscopy, flow cytometry, and fluorescence microplate reader platforms for live-cell pH and tracer studies.

AT A GLANCE

Protocol Summary

1. Prepare cells in a growth medium
2. Replace the medium with SNARF-1-Dextran Conjugate loading solution (100 µL/well for 96-well plate)
3. Incubate at 37°C for 5–20 minutes
4. Read Fluorescence at Ex/Em = 576/640 nm

KEY PARAMETERS

Fluorescence microscope

Emission	576 nm (pH 9)
Excitation	640 nm (pH 9)
Recommended plate	Black wall/clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit: https://www.aatbio.com/resources/guides/cell-samplepreparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Assay Protocol for Endocytosis

The following protocol is recommended for standard cell loading. It serves as a general guideline and may be adapted to suit specific experimental requirements.

1. Prepare cells as desired:

1. For example, plate adherent cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for a 96-well plate or 10,000 to 20,000 cells/well/25µL for 384-well plates.

   Note: Optimal cell density may vary by cell line and should be determined experimentally.

2. Prepare SNARF-1-dextran conjugate loading solution:

1. Prepare a 1mg/mL stock solution of SNARF-1-dextran conjugate in 1 mL of sterile water or Hanks and 20 mM HEPES buffer (HHBS). The stock solution should be used promptly. Any unused solution need to be aliquoted and refrozen at < -20°C.

   Note: Avoid repeated freeze-thaw cycles, and protect from light.
2. Prepare a 20-100 µg/mL SNARF-1-dextran conjugate loading solution in HHBS.

3. Run endocytosis assay:

1. Remove the medium, and add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) SNARF-1-dextran conjugate loading solution into the cell plate (from Step 2.2).

   Note: It is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate before dye-loading) if your compounds interfere with the serum.

   Note: Rapid trafficking of SNARF-1-dextran conjugate from early endosomes to late endosomes and subsequent fusion with lysosomes can occur. To aid the visualization of SNARF-1-dextran conjugate within the endosomes, we recommend increasing the labeling concentration and decreasing the loading time, and imaging immediately.
2. Incubate the dye-loading plate at cell incubator for 5 to 20 minutes.
3. Wash and replace the dye-loading solution with HHBS or growth medium.
4. Run the endocytosis assay by monitoring the fluorescence at Ex/Em (at pH9) = 576/640 nm.

Notes:

- SNARF-1 exhibits pH-dependent spectral properties. The optimal detection wavelength(s) may vary depending on the extent of endosomal and lysosomal acidification. Users should optimize fluorescence detection settings, dye-loading concentration, and incubation time for their specific cell type and instrument configuration.
- The fluorescence signal is relatively stable for at least one hour after trafficking to lysosomes.
- Because lysosomes have a lower pH than endosomes, lysosomal staining is typically brighter. Modulation of endocytosis or lysosomal function can be inferred by changes in fluorescence intensity.

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