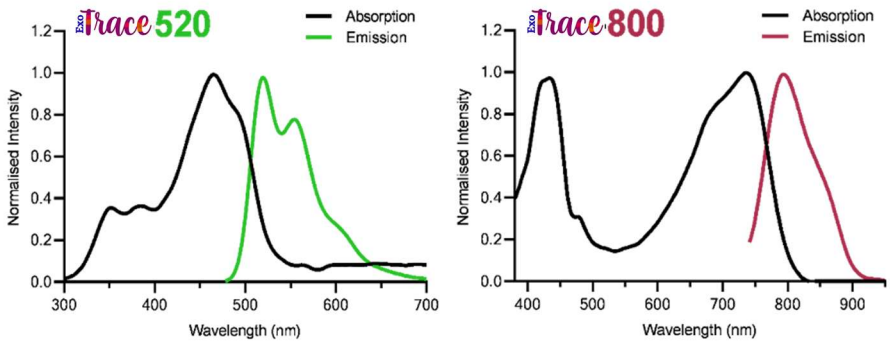
Technical Datasheet

ExoTrace for Membrane Labelling

Introduction

ExoTrace are a family of conjugated oligoelectrolytes (COEs) that intercalate into lipid bilayers. These lipophilic dyes fully embed themselves across the lipid bilayer of their target membranes, minimising dye dissociation or "leakage". ExoTrace are water-soluble and do not form micelles, thereby reducing the risk of false positive signals. They are also fluorogenic, exhibiting maximum fluorescence only upon intercalation into a lipid bilayer.

Exsure also offer exosome isolation kit compatible with ExoTrace along with reference *liposomes designed as positive and negative controls for fluorescence-based analyses of* nanoparticles such as extracellular vesicles (EVs). These include pre-stained fluorescent liposomes and unstained liposomes, which may be stained alongside biological samples to provide a reliable reference.



Absorption (UV-Vis Spectrometer) and Emission (Fluorometer) spectra were obtained for the ExoTrace in SUVs

Product	ExoTrace-520	ExoTrace-800
Appearance	Orange	Green
$\lambda_{abs\,(SUV)}$ (nm)	465	735
$\lambda_{em\,(SUV)}$ (nm)	511-530	775 - 818
Recommended Lasers	Blue (488 nm)	Red (638 nm)
Quantum Yield (%)	76	13.8
Fluorescence Lifetime (ns)	1.3	1.6
IC_{50} (µM)	>128	>272

Storage Conditions

Dye State	Storage	Shelf Life
Unopened dry dyes	2 to 8°C, protected from light	Refer to expiry date on vial
Reconstituted dyes	2 to 8°C, protected from light	Up to 1 month
Frozen reconstituted dyes	-80°C, protected from light	Up to 3 months, avoid freeze-thaw cycles

Dye Reconstitution

Ensure that your fluorescence-based instruments are equipped with the appropriate lasers and detectors for the chosen dyes before proceeding with your experiment.

I. Allow all buffers and reagents to come to room temperature before use.

II. To get a 25 µM stock solution, reconstitute the dried dye in 100 µL of filtered 1X PBS or water.

III. Vortex, then perform a quick spin to collect the dye stock solution at the bottom of the tube.

IV. Sonicate the dye stock solution at 40°C for 15 minutes.

V. Repeat Step 3.

VI. Dyes perform best when freshly reconstituted. While reconstituted dyes can be stored for up to 1 month at 2 to 8°C, perform Steps 3 to 5 prior to using previously reconstituted dyes.

EV Sample Preparation

VII. Dilute EV to 1e10 particles/mL using a filtered buffer of your choice.

VIII. Dilute a suitable volume of 25 µM stock solution to 10 µM in a clean Eppendorf tube, using the same filtered buffer. *(e.g. 6 µL of 25 µM dye stock + 9 µL of filtered buffer)*

IX. Incubate 1e10 particles/mL diluted EV with 1 µM* final dye concentration for 1 hour at 37°C, protected from light. *(e.g. 5 µL of 10 µM diluted dye solution + 45 µL of 1e10 particles/mL EV)*

If an incubator is not available, incubate the stained samples at room temperature.

It is essential to optimise the staining concentration for your EV type, dye and instrument by performing a titration. Suggested final dye concentrations are 0.1 µM, 0.5 µM, 1 µM, 1.5 uM, 2 uM.

X. Include the corresponding controls to identify background signals:

- Dye Only *(e.g. 5 µL of 10 µM diluted dye solution + 45 µL of filtered buffer)*
- EV Only *(e.g. 5 µL of filtered buffer + 45 µL of 1e10 particles/mL EV)*
- Buffer Only

For Flow Cytometry:

a. As a starting point, dilute stained EV sample 100X using a filtered buffer of your choice.

b. Acquire the 100X diluted sample and collect data as per instrument's user guide.

c. Optimise the dilution of the stained EV sample to achieve instrument manufacturer's recommended nanoparticle acquisition parameters (e.g. abort rate, event count).

Each flow cytometer requires an optimal event rate to avoid:

a) *Swarming from overly concentrated samples,*
b) *Very weak signals from excessively diluted ones.*

E.g. The recommended event rate for stained EV samples is 1,000-1,500 events/second, the dilution factor to achieve this should be optimised for specific sample types.

d. Apply this optimised dilution factor uniformly to all related samples, including controls.

e. Use the same instrument settings (gain, threshold and width) and acquisition time across all samples to ensure reliable comparisons.

For Plate Reader:

Prepare samples with reaction volume 100 µL per well in a 96-well polystyrene non-tissue culture treated plate (clear flat bottom), avoiding the outer wells of the plate. After incubation, measure the full fluorescence spectrum or fluorescence at peak maxima.

Important Guidelines for EV Labelling with ExoTrace

- Filter all buffers through a 0.2 µm filter before use.
- Sonicate, vortex and spin down the ExoTrace (Steps 3 to 5) before every use to ensure low background in Dye Only controls.
- EVs perform best when used fresh after isolation or preparation. Make a fresh 1e10 particles/mL dilution on each test day, as particle counts tend to drop over time.
- Each ExoTrace has its own unique brightness and staining efficiency. Titrate and optimise the labelling concentration for each dye, EV type, study condition and instrument.
- To improve staining efficiency, it may be helpful to incubate the EV sample with the dye for a longer duration (e.g. 2 hours - overnight).
- Consider purifying the EV sample using Exosure to get the optimum results