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Quick Start Guide

Aco-Dyes™ for Labelling Extracellular Vesicles

(A) Dye Reconstitution

1 Add 100µL of filtered 1X PBS / water

Aco-Dye 100 Tests Size

Photophysical Properties of Aco-Dyes

Product	Aco-430™	Aco-490™	Aco-520™	Aco-600™	Aco-650™	Aco-800™
$\lambda_{abs\ (SUV)}$ (nm)	369	422	465	525	632	735
$\lambda_{em\ (SUV)}$ (nm)	403-460	458-508	511-530	586-635	645-661	775-818
Recommended Laser(s)	UV (355nm)	Violet (405nm)	Blue (488nm)	Blue/Yellow (488/561nm)	Red (638nm)	Red (638nm)

Ensure that your instrument is equipped with the appropriate lasers and filters to detect the chosen dye(s) before proceeding with your experiment.

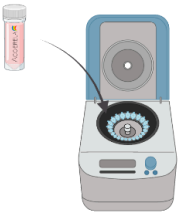
Storage Conditions

- Dry Dyes: Room Temperature
  Refer to expiry date on tube
- Reconstituted Dyes: 2-8°C
  Up to 1 month
- Frozen Reconstituted Dyes: -80°C
  Up to 3 months; avoid freeze-thaw

Aco-Dyes perform best when freshly reconstituted. Perform Steps 2 to 5 prior to using previously reconstituted dyes.

2 Vortex to mix

3 Quick spin



[Important!]

4 Sonicate at 40°C for 15 minutes

If a sonicator is not available, incubate at room temperature for 15 minutes.

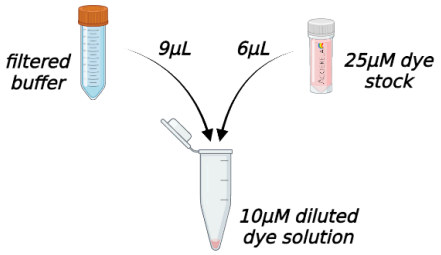
5 Repeat Steps 2-3: Vortex > Quick spin

25µM dye stock solution

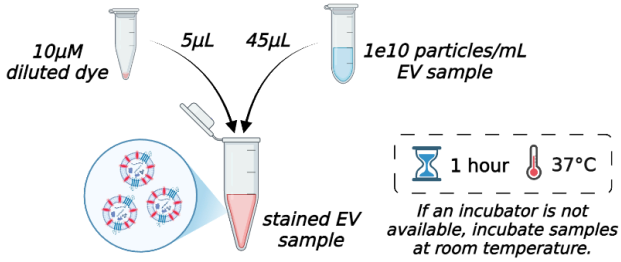
(B) EV Labelling for Flow Cytometry

**It is essential to optimise the staining concentration for your EV type, dye and instrument.*

6 Dilute dye stock to 10µM
e.g. 9µL buffer + 6µL dye



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

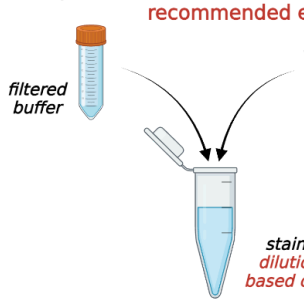


If an incubator is not available, incubate samples at room temperature.

Important Considerations

1. It is recommended to include the following controls to identify background signals and rule out false positives.
   - Dye Only *[same dye conc as stained EV]*
     e.g. 45µL buffer + 5µL of 10µM dye
   - EV Only
     e.g. 45µL of 1e10 particles/mL EV + 5µL buffer
   - Buffer Only
2. The dilution factor optimised in Step 8 for the stained EV samples should be applied uniformly to all related samples, including controls.
3. Using the same instrument settings (gain, threshold and width) and acquisition time across all samples ensures reliable comparisons.

8 Dilute stained EV samples just before flow cytometry acquisition to achieve instrument manufacturer's recommended event rate



stained EV sample diluted 1:100, dilution factor should be optimised based on flow cytometer and EV type.