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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-800™ |
|---|---|---|---|---|---|
| $\lambda_{abs\ (SUV)}$ (nm) | 369 | 422 | 465 | 525 | 735 |
| $\lambda_{em\ (SUV)}$ (nm) | 403-460 | 458-508 | 511-530 | 586-635 | 775-818 |
| Recommended Laser(s) | UV (355nm) | Violet (405nm) | Blue (488nm) | Blue/Yellow (488/561nm) | 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.*

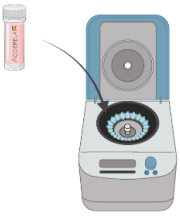
**Storage Conditions**

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

*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.*

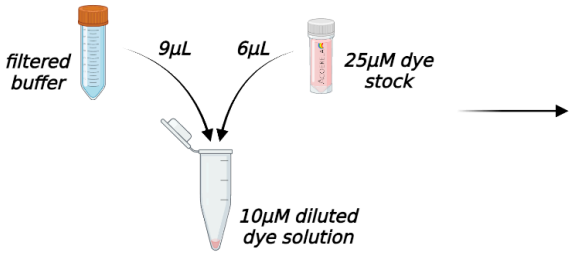
**5** Repeat Steps 2-3: Vortex > Quick spin

*25µM dye stock solution*

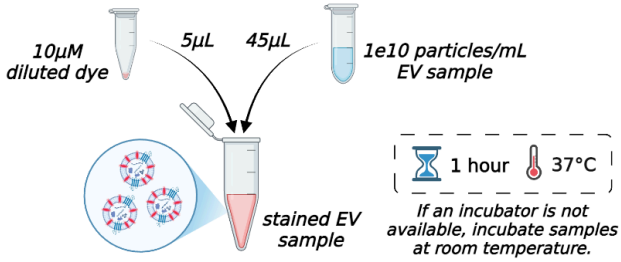
## (B) EV Labelling for Flow Cytometry

**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*

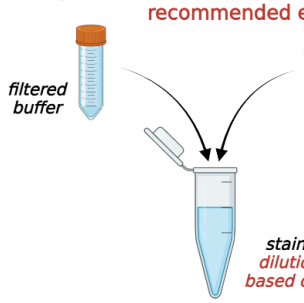



*If an incubator is not available, incubate samples at room temperature.*

**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.*

*Version 1.0 (Updated Oct 2025)*