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Frequently Asked Questions

Aco-Dyes™ for Membrane Labelling, AcoRL™ Reference Liposomes

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GENERAL INFORMATION

1. What is the molecular weight and mass of Aco-Dye in each vial?

Aco-Dyes	Molecular Weight (g/mol)	Mass of Dye in 100T Vial (µg)
Aco-430™	2015.8	5.04
Aco-490™	2027.8	5.07
Aco-520™	2082.9	5.21
Aco-600™	2069.8	5.17
Aco-800™	1883.9	4.71

2. What are the recommended storage conditions for Acoerela's products?

Acoerela's products are shipped at ambient temperature but should be stored according to the guidelines in the table and protected from light.

Storage Conditions for Aco-Dyes	
Unopened dry dyes	2 to 8°C; Refer to expiry date on vial.
Reconstituted dyes	2 to 8°C; Up to 1 month.
Frozen reconstituted dyes	-80°C; Up to 3 months, avoid freeze-thaw cycles.
Storage Conditions for AcoRL Reference Liposomes	
Unopened lyophilised liposomes	Room temperature; Refer to expiry date on vial.
Reconstituted liposomes	2 to 8°C; Up to 3 months.

3. Can I freeze Acoerela's products for long-term storage?

Frozen reconstituted Aco-Dyes remain stable for up to 3 months when stored at -80°C. We recommend aliquoting the product to avoid freeze-thaw cycles.

Do not freeze AcoRL Reference Liposomes.

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DYE RECONSTITUTION

4. What buffers can I use for reconstitution?

For Aco-Dyes, we recommend using filtered 1X PBS or water. If required, you may also use an aqueous buffer of your choice without additives like BSA or detergents.

For AcoRL Reference Liposomes, reconstitute the product using filtered water.

5. Is sonication of Aco-Dyes really necessary?

Sonication is **highly recommended** for nano-based experiments to ensure the Aco-Dye is fully solubilised. If you do not have access to a sonicator, allow the reconstituted Aco-Dye to equilibrate to room temperature for at least 15 minutes before vortexing the solution and performing a quick spin.

6. What other solvents are the Aco-Dyes soluble in?

Aco-Dyes are also soluble in DMSO, ethanol and methanol.

SAMPLE PREPARATION

7. How much Aco-Dye should I use for staining?

As a starting point, we suggest using 1 µM of Aco-Dye to stain 1e10 particles/mL of extracellular vesicles (EVs). However, it is **essential to perform dye titration** (e.g. 0.1 µM, 0.5 µM, 1 µM, 1.5 uM, 2 uM) in your initial experiments to optimise the staining concentration for your EV type. Refer to our Technical Datasheet for the detailed EV staining protocol.

8. How long should I stain with Aco-Dyes?

We recommend staining for 1 hour at 37°C. If an incubator is not available, staining at room temperature is also possible. Staining at 4°C is suitable for longer incubations if desired.

9. Do you provide controls?

Yes. For positive controls, we recommend using AcoRL Reference Liposomes — dye-matched liposomes (particle size 50-100 nm) that come pre-stained with the corresponding Aco-Dye. We also provide a negative control — AcoRL-Neg, an unstained liposome, which also gives you the flexibility to stain them yourself.

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10. Which types of extracellular vesicles (EVs) have been stained with Aco-Dyes?

Source	Extracellular Vesicle	Method of Detection
Animal	Cow Milk	Flow Cytometry
	Ovine Fetal Cells*	Flow Cytometry, Confocal Microscopy, Live Cell Imaging
Bacteria	*E. coli*	Flow Cytometry
Human	A549 Cell Line	Flow Cytometry
	Adipose-Derived Stem Cells (ASC)*	Flow Cytometry, Confocal Microscopy
	Human Embryonic Kidney (HEK) Cells	Flow Cytometry
	Mature Osteoclast-Conditioned Medium (OC-CM)	Flow Cytometry
	Pancreatic Cells	Flow Cytometry
	PC-3 Cell Line	Flow Cytometry
	Plasma*	Flow Cytometry
	Platelet	Flow Cytometry
	Red Blood Cells (RBC) (ex vivo*; in vitro)	Flow Cytometry, Confocal Microscopy
	Serum*	Flow Cytometry
	Umbilical Cord Mesenchymal Stem Cells (MSC)	Flow Cytometry
Plant	Aloe Vera	Flow Cytometry
	Coconut	Flow Cytometry
	Dendrobium	Flow Cytometry
	Ginseng	Flow Cytometry
	Houttuynia	Flow Cytometry

**Customer reported*

11. Can I co-stain with other probes, such as conjugated antibodies or CFSE?

Yes. Refer to our Application Note on co-staining extracellular vesicles (EVs) with tetraspanin markers and Aco-Dyes.

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12. How can I remove excess Aco-Dye post-staining?

You can use the Amicon® Ultra Centrifugal Filter or dialysis; both methods work well with a 100 kDa MWCO (Molecular Weight Cut-Off). Alternatively, you can use the Vesi-SEC micro Size Exclusion Chromatography spin-column from Vesiculab.

13. Can you advise on the signal stability/retention of the Aco-Dyes?

From our internal stability tests, we found that our AcoRL Reference Liposomes, which come pre-stained with Aco-Dyes, remain stable for 3 months with a slight signal drop observed at the 6-month timepoint. This kind of stability can be attributed to the dyes' full intercalation and ability to span the entire lipid bilayer membrane unlike traditional hairpin-structured membrane dyes, which bind only to a small region of the membrane and may easily dissociate over time.

In this Publication, one of our non-commercial dyes was used for long-term in vivo tumor tracking in mice and showed no signal loss for 26 days, which was the end of the study. This gives a good idea on how long the Aco-Dyes can retain their signal in vivo.

14. Does the signal stability of the Aco-Dyes depend on the integrity of the EV membrane?

Aco-Dyes bind to the lipid bilayer of the EV membrane. If the membrane ruptures after dye intercalation, the outcome depends on how the breakage occurred. Membrane fragments that retain the dye may continue to fluoresce.

15. Can I fix my stained samples?

Since fixation disrupts the membrane, it impacts the retention of the Aco-Dyes within the lipid bilayer. However, this can be slowed down by optimising the fixation time, incubation and storage conditions, and storage duration prior to visualisation.

In this Publication, following EV uptake, cells were fixed with 4% formalin for 10 minutes and permeabilised with 0.2% Triton X-100 for 20 minutes before confocal microscopy imaging.

INSTRUMENT SPECIFICATIONS

IMPORTANT NOTE: Ensure that your fluorescence-based instruments are equipped with the appropriate lasers and detectors for the chosen dye(s) before proceeding with your experiment. Feel free to reach out to us at hello@acoerela.com for technical support.

16. Which laser(s) can be used for excitation?

Products	Recommended Lasers	Other Possible Lasers
Aco-430™, AcoRL-430™	355nm (UV)	-

Products	Recommended Lasers	Other Possible Lasers
Aco-490™, AcoRL-490™	405nm (violet)	488nm (blue)
Aco-520™, AcoRL-520™	488nm (blue)	-
Aco-600™, AcoRL-600™	488nm (blue), 561nm (yellow green)	-
Aco-800™, AcoRL-800™	638nm (red)	405nm (violet)

17. Which instruments have Aco-Dyes been tested on?

This list provided is not exhaustive. For other instruments not listed here, we encourage you to contact us at hello@acoerela.com for technical support.

Vendor	Instrument
Flow Cytometry	
Beckman Coulter	CytoFLEX LX
	CytoFLEX nano
	CytoFLEX S
	CytoFLEX SRT Cell Sorter
BD Biosciences	FACSCanto™ II
	FACSymphony™ A1
	LSRFortessa™
Cytek	Amnis® ImageStream®X Mk II
	Aurora™
Kinetic River	Delaware Flow NanoCytometer®
NanoFCM	Flow NanoAnalyzer
Nanoparticle Tracking Analysis (NTA)	
Particle Metrix	ZetaView®
Confocal Microscopy	
Nikon	ECLIPSE Ti2-E
Live-Cell Imaging	
Sartorius	IncuCyte® S3 Kinetic Imaging System

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UNEXPECTED RESULTS

18. Why am I observing a non-specific signal in my Dye Only control?

Each flow cytometer requires an optimal event rate to avoid
(a) swarming from overly concentrated samples,
(b) very weak signals from excessively diluted ones.

Stained samples should be diluted to achieve the instrument manufacturer's recommended nanoparticle acquisition parameters such as the abort rate and/or event count. For example, the recommended event rate is 1,000-1,500 events/second for the CytoFLEX nano, and 2,000-10,000 events/µL for the CytoFLEX LX and NanoFCM Flow NanoAnalyzer).

This dilution factor, optimised for specific sample types, should be applied uniformly to all related samples including controls. Instrument settings (gain, threshold and width) and acquisition time should also be kept constant across all samples to ensure reliable comparisons.

19. Why does Aco-490 appear in the CytoFLEX nano B531 channel?

Due to the collinear laser design of the CytoFLEX nano, the Aco-490 signal appears strongly in the B531 channel rather than the expected V447 channel. In contrast, the Aco-490 fluorescence signal appears strongly in the expected violet channel with minimal spillover into the blue channel when run on other flow cytometers of the CytoFLEX series such as the CytoFLEX LX or CytoFLEX S, as these instruments have spatially separated laser paths.

OTHER DYE APPLICATIONS

20. Have the Aco-Dyes ever been used in in vivo models?

Yes. Refer to the Publications Section for further details.

21. Can Aco-Dyes be used for labelling lipid nanoparticles (LNP)?

Yes. Refer to this Publication.

22. Can Aco-Dyes be conjugated to antibodies?

No.

23. Can Aco-Dyes cross the blood brain barrier?

Yes.