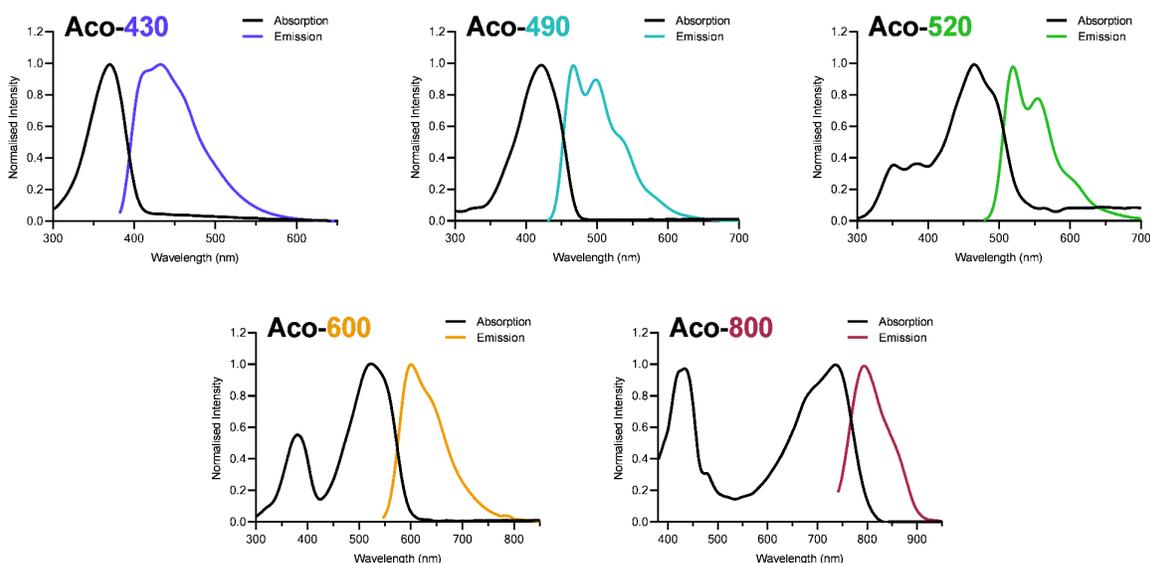


Technical Datasheet **AcoRL™ Reference Liposomes**

Introduction

Acoerela's **AcoRL Reference Liposomes** are pre-stained fluorescent liposomes, carefully formulated by combining a blend of biologically relevant synthetic lipids, along with 1 mol% Aco-Dyes™ relative to total lipid content. Designed to support the optimisation of instrument parameters, these liposomes provide a reliable reference for fluorescence-based analyses of nanoparticles such as extracellular vesicles (EVs). Available as both positive and negative controls with particle size ranging from 50-100 nm*, AcoRL Reference Liposomes help researchers visualise and analyse Aco-Dye-stained biological samples with precision using flow cytometry or other fluorescence-based instruments.

**Refer to lot-specific Certificate of Analysis (COA) for exact particle size, particle concentration and polydispersity index.*



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

Product	AcoRL-430™	AcoRL-490™	AcoRL-520™	AcoRL-600™	AcoRL-800™	AcoRL-Neg™
Fluorophore	Aco-430™	Aco-490™	Aco-520™	Aco-600™	Aco-800™	Unstained
Appearance	Colourless	Yellow	Orange	Purple	Green	Colourless
λ_{abs} (nm)	369	422	465	525	735	-
λ_{em} (nm)	403 - 460	458 - 508	511-530	586 - 635	775 - 818	-
Recommended Lasers	UV (355 nm)	Violet (405 nm)	Blue (488 nm)	Blue / Yellow (488 / 561 nm)	Red (638 nm)	-
Quantum Yield (%)	94	60	76	27	13.8	-
Fluorescence Lifetime (ns)	1.1	0.9	1.3	1.9	1.6	-

Storage Conditions

Liposome State	Storage	Shelf Life
Unopened lyophilised liposomes	Room temperature, in original vacuum packaging	Refer to warranty statement below
Reconstituted liposomes	2 to 8°C, protected from light	Up to 3 months

Liposome Reconstitution

Ensure that your fluorescence-based instruments are equipped with the appropriate lasers and detectors for the chosen Aco-Dyes and/or AcoRLs before proceeding with your experiment.

1. Allow all buffers and reagents to come to room temperature before use.
2. Resuspend AcoRL in 100 µL of room temperature filtered water.
3. Vortex for 10 seconds at medium intensity to disperse the particles evenly.
4. Reconstituted liposomes can be stored for up to 3 months at 2 to 8°C, protected from light. Prior to each use, allow the reconstituted liposomes to equilibrate to room temperature for 1 hour before vortexing (Step 2).

Detection via Flow Cytometry

5. As a starting point, dilute the reconstituted liposomes 10,000X using a room temperature filtered buffer of your choice.
6. Acquire the 10,000X diluted liposomes and collect data as per instrument's user guide.
7. Optimise the dilution of reconstituted liposomes according to the instrument manufacturer's recommended nanoparticle acquisition parameters (e.g., abort rate, event count) to avoid swarming effect and ensure a uniform distribution. This will help achieve clear, well-defined flow cytometry plots and reliable data.
8. Upon optimising the dilution of reconstituted liposomes, acquire and adjust instrument settings as necessary.

Technical Support

FAQs and relevant publications can be found on the Support page of our website www.acoerela.com. Feel free to reach out to us at hello@acoerela.com for technical support and troubleshooting.

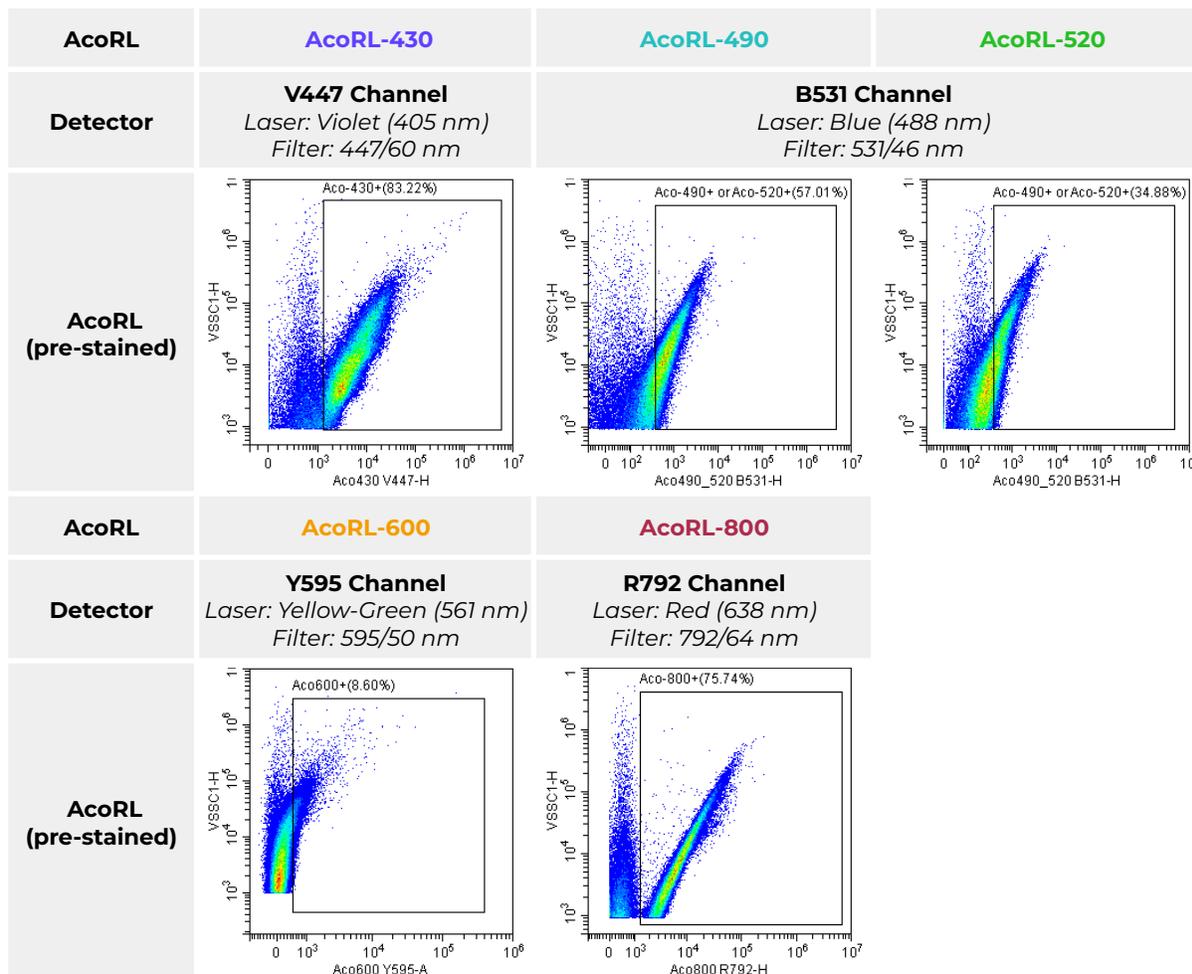
Warranty Statement

Acoerela warrants that, for twenty four (24) months from the date of manufacture by Acoerela, the Product will perform in accordance with the specifications described in this datasheet when used and stored as set forth in this datasheet. If it is proven to the satisfaction of Acoerela that the Product fails to meet these specifications, Acoerela will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product.

Acoerela’s liability is expressly limited to replacement of the non-conforming Product or, at Acoerela’s election, a refund limited to the actual purchase price paid by the Customer for the non-conforming Product. Warranty claims may only be made within the warranty period. Acoerela may request a preliminary investigation of reported Product non-conformance by the Customer. Acoerela may also require that the Product subject to a warranty claim be returned for inspection and Acoerela may test performance of retained samples of Product to verify non-conformance. Acoerela’s liability does not extend to any damages arising from improper use or storage of the Product, or losses associated with the use of additional materials or reagents not mentioned in this datasheet. Acoerela does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose or non-infringement.

Data and Figures

Figure 1. **AcoRL-430**, **AcoRL-490**, **AcoRL-520**, **AcoRL-600** and **AcoRL-800** were acquired on the **Beckman Coulter CytoFLEX nano Flow Cytometer**. The samples were collected at an event rate of 1,000-1,500 events/second.



NOTES:

1. 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.
2. **Aco-600** signal detection is suboptimal on the CytoFLEX nano flow cytometer.

Figure 2. AcoRL-430, AcoRL-490, AcoRL-520, AcoRL-600 and AcoRL-Neg were acquired on the Beckman Coulter CytoFLEX LX Flow Cytometer configured with VSSC, at slow flow rate. The samples were collected at an event rate of 2,000-10,000 events/ μ L.

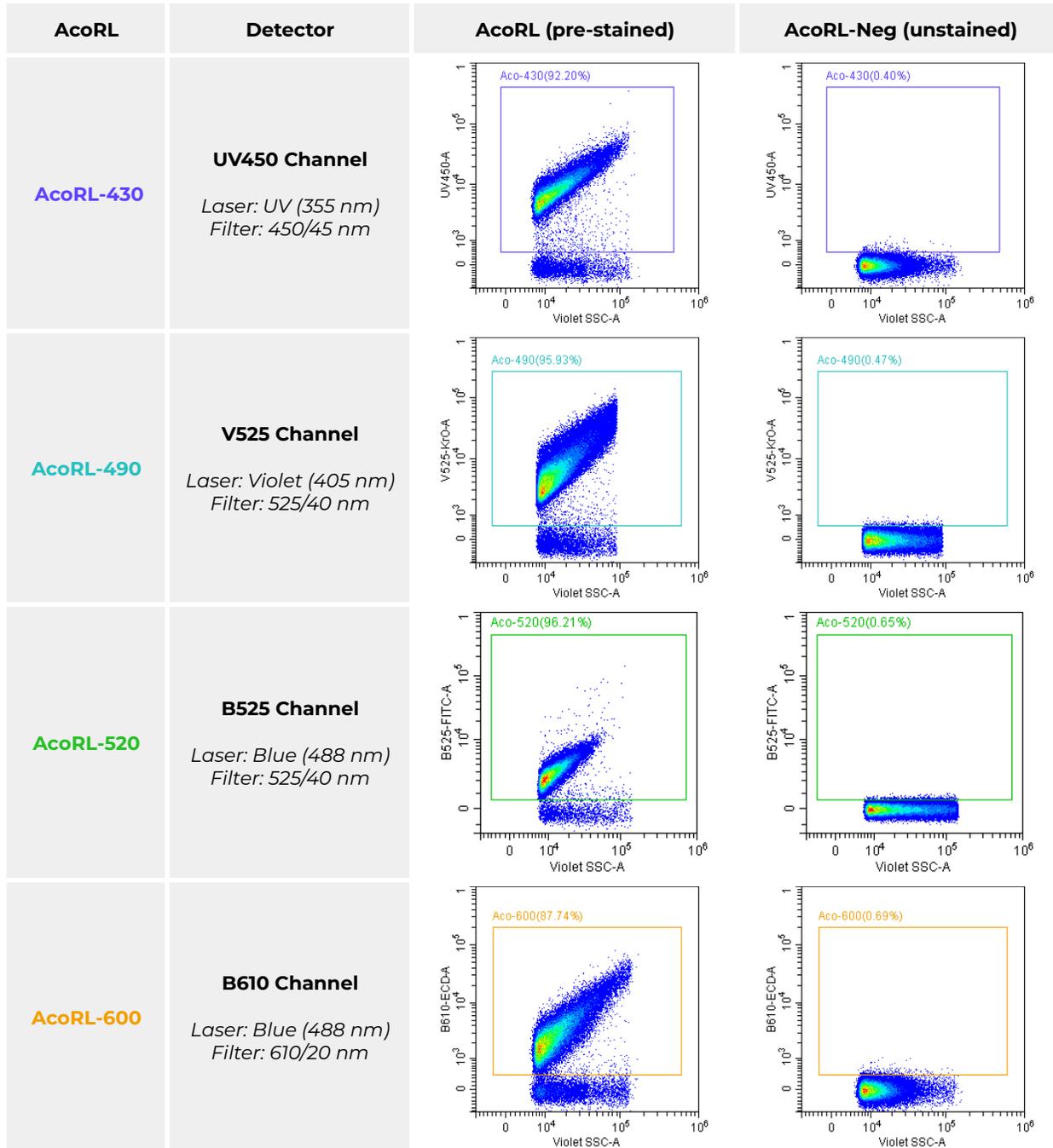


Figure 3. AcoRL-520, AcoRL-600, AcoRL-800 and AcoRL-Neg were acquired on the NanoFCM Flow NanoAnalyzer calibrated using small beads as per manufacturer's instructions. The samples were collected at an event rate of 2,000-10,000 events/ μ L.

NOTE: To combat stickiness of samples, **1X TAE buffer** can be used in place of 1X PBS for diluting liposomes before acquisition.

