

# CAMAG Derivatizer

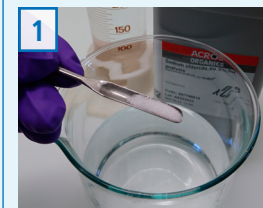
## Recommendations for common derivatization reagents

Derivatization reagent	Use	Transfer from manual to automated spraying			Transfer from immersion to automated spraying	
		Nozzle	Spraying level 20 × 10 cm, 2 mL	Spraying level 20 × 20 cm, 4 mL	Nozzle	Spraying level 20 × 10 cm, 3 mL
10% sulfuric acid reagent	Spray, then heat the plate at 100°C for 3 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under UV 366 nm and white light. (Please note that sulphuric acid > 20% in methanol cannot be sprayed.)	yellow	3–4	4–5	blue	3–4
p-anisaldehyde – sulfuric acid reagent	Spray, then heat the plate at 100°C for 3 min on the CAMAG Plate Heater, let cool to room temperature. Detection under UV 366 nm and white light.	blue	3–4	4–5	blue	1–3
NP reagent	100 °C for 3 min, let cool to room temperature, spray, wait 5 min. Detection under UV 366 nm	green	3–4	4–5	green	3–4
PEG solution	Spray, wait 5 min. Detection under UV 366 nm.	blue	2–3	3–4	green	4–5
Iodine solution	Spray, dry with cold air for 2 min. Detection after background has turned white again. Detection under UV 254 nm and white light.	blue	3–4	4–5	green	6
Dragendorff's reagent	Spray, dry with cold air for 10 min. Detection under white light.	red	2–3	3–4	red	3–4
Fast blue salt B reagent	Spray. Detection under white light within 2 min after spraying (white background).	green	3–4	4–5	green	3–4
Ehrlich's reagent	Spray, heat the plate at 100°C for 5 min on the CAMAG TLC Plate Heater, and let cool to room temperature. Detection under white light.	yellow	5–6	6	blue	1–2
Phosphomolybdic acid reagent	Spray, heat at 120°C for 10 min on the CAMAG TLC Plate Heater, and let cool down to room temperature. Detection under white light.	yellow	6	6	yellow	6
Ninhydrin reagent	Spray, heat at 105°C for 3 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under white light.	blue	3–4	4–5	green blue	6 3–5
Copper (II) sulfate reagent	Spray, heat the plate at 110°C for 10 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under white light.	blue	3–4	4–5	blue	5–6
Aniline – diphenylamine – phosphoric acid reagent	Spray, heat at 110°C for 10 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under white light.	yellow	5–6	5–6	yellow	6
Vanillin – sulfuric acid reagent	Spray, heat at 100°C for 3 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under UV 366 nm and white light.	yellow	3–4	4–5	yellow	2–3
Potassium hydroxide solution*	Spray, heat at 100°C for 2 min on the CAMAG TLC Plate Heater, let cool to room temperature. Detection under UV 366 nm and white light.	blue	3–4	4–5	green blue	2–3 3
Enzymatic test: Tyrosinase (enzyme and substrate in aqueous solutions)	Spray subsequently the appropriate volume of substrate solution and the appropriate volume of enzyme solution onto the plate. Incubate the plate for 10 minutes at room temperature in a closed box to prevent from drying (e.g. inside of the glass covered drawer of the BioLuminizer). Dry the plate to <2% relative humidity for 5 minutes in a desiccator or in the ADC 2 by using molecular sieve.	yellow	(3 mL) 4–5	n/a	yellow	4–5

Recommendation to obtain optimal results: 20°C < T < 25°C and 35% < relative humidity < 45%

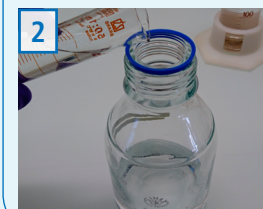
\*) Recommended to use with the chemically resistant Ultra nozzle

### Preparation of cleaning solution



Dissolve half of a spatula (~ 300 mg) of sodium chloride (NaCl) in 500 mL water.

The concentration **does not have to be analytically accurate**.

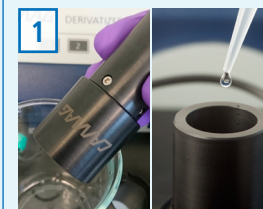


Dilute 10 mL of the aqueous NaCl solution in 90 ml ethanol.

(Alternatively, in 90 ml ethanol : methanol 1:1 or in any other solvent that can be sprayed and dissolves residues in the nozzle)

This results in a ~ 1 mmol/L NaCl cleaning solution.

### Cleaning of the nozzle



Empty the nozzle, and spray 2 mL of cleaning solution (level 6) before / after each spraying.

(In case spraying is not possible with this solution, add ~ 50 % methanol to the cleaning solution).



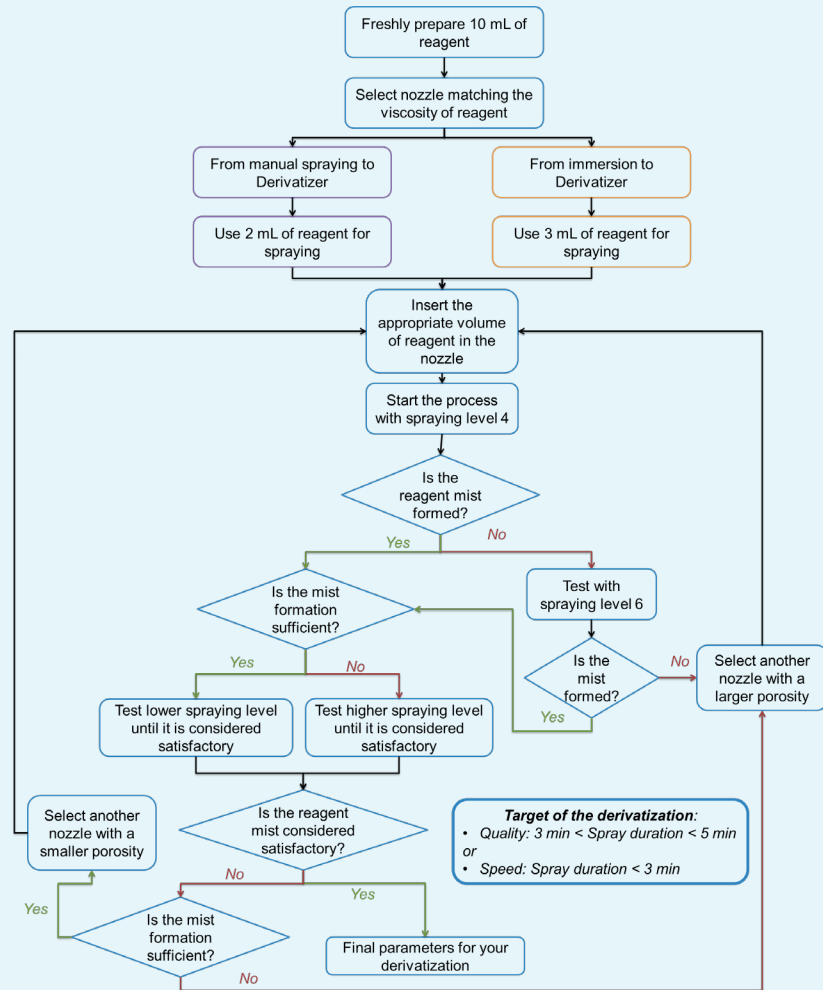
When necessary or when changing spraying reagent: **rinse the tip of the nozzle** and the inside of the reagent container with water, ethanol or cleaning solution.

Do not rinse the entire nozzle as this might harm the electrical connection.

**The nozzle has to be dry before use.**

# CAMAG Derivatizer

## Transfer from manual spraying or immersion to automated spraying



low  $\xrightarrow{\text{Viscosity of the derivatization reagent}}$  high  
 Green < Blue < Yellow < Red

short  $\xrightarrow{\text{Spraying time}}$  long  
 Level 6 < 5 < 4 < 3 < 2 < Level 1

## Preparation of derivatization reagents\*

### Aniline-diphenylamine-phosphoric acid reagent (for spraying)

Dissolve 2 g of diphenylamine and 2 mL of aniline in 80 mL of methanol. After addition of 10 mL of *o*-phosphoric acid (85%), fill up to 100 mL with methanol.

### Aniline-diphenylamine-phosphoric acid reagent (for immersion)

Dissolve 4 g of diphenylamine in 160 mL of acetone, add 4 mL of aniline, and carefully add 30 mL of *o*-phosphoric acid. Shake well to dissolve the initially formed precipitate.

### *p*-Anisaldehyde sulfuric acid reagent (for spraying and immersion)

Place 85 mL of methanol in a 100 mL glass bottle and cool it down in a water-ice cubes-salt bath or in a freezer. To the ice-cold methanol add slowly and carefully 10 mL of acetic acid and 5 mL of sulfuric acid and mix well. Allow the mixture to cool to room temperature, then add 0.5 mL of *p*-anisaldehyde.

### Copper(II) sulfate reagent (for spraying)

Dissolve 1.5 g of copper(II) sulfate pentahydrate in a few milliliters of water and fill up to 100 mL with methanol.

### Dragendorff's reagent (for spraying)

Solution A: Weigh 0.85 g of basic bismuth nitrate in a glass bottle and add 10 mL of glacial acetic acid and 40 mL of water.  
 Solution B: Weigh 8 g of potassium iodide in a glass bottle and dissolve in 30 mL of water.  
 Just before spraying, mix 1 mL of solution A and 1 mL of solution B and 4 mL of acetic acid in 20 mL water.

### Ehrlich's reagent (for spraying)

Dissolve 0.5 g of 4-dimethylaminobenzaldehyde in 150 mL of methanol, and add 50 mL of hydrochloric acid (37%).

### Fast blue salt B reagent (for spraying and immersion)

Dissolve 250 mg of fast blue salt B (*o*-dianisidine bis(diazotized) zinc double salt) in 10 mL of water and mix with 25 mL of methanol and 15 mL of dichloromethane. Prepare fresh on each day.

### Iodine solution (for spraying)

Place 0.5 g of iodine in a glass bottle and dissolve in 100 mL of ethanol. Store in a dark place.

### Natural products reagent (NP reagent) (for spraying)

Dissolve 1.0 g of 2-aminoethyl diphenylborinate in 100 mL of methanol.

### Natural products reagent (NP reagent) (for immersion)

Dissolve 1.0 g of 2-aminoethyl diphenylborinate in 200 mL of ethyl acetate.

\* To obtain comparable results to manual spraying or to immersion

### Ninhydrin reagent (for spraying)

Dissolve 0.1 g of ninhydrin (2,2-dihydroxyindene-1,3-dione) in 50 mL of ethanol (96%) and add 1.5 mL of glacial acetic acid.

### Ninhydrin reagent (for immersion)

Dissolve 0.6 g of ninhydrin (2,2-dihydroxyindene-1,3-dione) in 190 mL of isopropanol and add 10 mL of glacial acetic acid.

### Potassium hydroxide solution (for spraying)

Dissolve 5 g potassium hydroxide in 100 mL of methanol (96%).

### Phosphomolybdic acid reagent (for spraying)

Dissolve 5 g of phosphomolybdic acid hydrate in 200 mL of ethanol (96%).

### Polyethylene glycol reagent (PEG reagent) (for spraying)

Dissolve 5 g of polyethylene glycol 400 (macrogol) in 100 mL of ethanol (96%).

### Polyethylene glycol reagent (PEG reagent) (for immersion)

Dissolve 10 g of polyethylene glycol 400 (macrogol) in 200 mL of dichloromethane.

### Sulfuric acid reagent (for spraying and immersion)

Dissolve 10 mL of concentrated sulfuric acid in 90 mL of methanol under cooling.

### Vanillin reagent R (for spraying and immersion)

Dissolve 1 g of vanillin in 100 mL of ethanol 96% and carefully add 2 mL of concentrated sulfuric acid. Use within 48h.

### Enzymatic assay: Tyrosinase (aqueous solution)

Preparation of phosphate buffer 0.02 M, pH = 6.8  
 Solution A: Dissolve 0.35 g of potassium phosphate dibasic ( $K_2HPO_4$ ) in 100 mL of deionized water (in a volumetric flask).  
 Solution B: Dissolve 0.28 g of sodium phosphate monobasic monohydrate ( $NaH_2PO_4 \cdot H_2O$ ) in 100 mL of deionized water (in a volumetric flask).

Mix 4 parts of solution A with 6 parts of solution B. Measure the pH of the solution. To adjust the pH to 6.8 add a few drops of solution A or B.

Preparation of the enzyme solution: Stock solution: Prepare a stock solution with an activity of 12'000 U/mL by dissolving the required amount of mushroom tyrosinase in phosphate buffer. Ten aliquots of 100  $\mu$ L each are made and stored at -20°C. Before use, an aliquot is diluted with 3 mL of phosphate buffer to reach an activity of 400 U/mL. (Example: 3.83 mg of tyrosinase (activity: 3130 U/mg) are dissolved in 1 mL of phosphate buffer 0.02 M, pH 6.8).  
 Substrate solution: L-DOPA 12 mmol/L: dissolve 0.047 g of L-DOPA in 20 mL of phosphate buffer containing 1% Triton X-100 and sonicate for 40 min. The solution can be used for maximum 3 days, if stored in the dark at 4°C.

## Further spraying reagents for the Derivatizer

CAMAG recommends spraying reagents that have been tested and can be sprayed without problems with the Derivatizer. For untested reagents, if unwanted dripping occurs or a reagent cannot be sprayed at all, diluting the mixture (preferably with methanol / ethanol) or changing the solvent might help.

## Mixtures that can be sprayed with the Derivatizer

**Substances that are not on our list of recommended spraying reagents but can be sprayed according to user feedback.** CAMAG did not test the mixtures or optimize the nozzle type and spraying level.

Reagent	Preparation / Recipe / Remarks
<b>A. fischeri suspension</b>	According to DIN EN ISO 11348-1, 2009 Nozzles: red / blue / yellow, spraying level 6
<b>Acetic anhydride / sulfuric acid reagents</b>	1 mL acetic anhydride in 10 mL methanol Nozzle: red, spraying level 6.
<b>Acetone</b>	Spraying reagents containing high concentration of acetone (< 90 %) can usually be sprayed without problems Nozzle: yellow
<b>AChE Inhibition Assay reagents</b>	0.5 mL Tris-HCl buffer solution (pH 7.8, 0.05 M)  AChE solution (6.66 U/mL)  1:1 substrate/chromogenic reagent mixture (ethanolic 1-naphthyl acetate solution and aqueous Fast Blue B salt solution, 3 mg/mL each)  All reagents: Nozzle green, spraying level 6  Other mix: 100 mL Tris-HCl buffer 0.05 M, pH 7.8 plus 666 units AChE (or 334 units BChE) and 100 mg bovine serum albumin Nozzle green
<b>Aminobenzoic acid Reagent</b>	100 mg of aminobenzoic acid dissolved in 1.8 mL acetic acid. Add 2 mL of water and 0.1 mL of o-phosphoric acid. Prior to use, add 6 mL of acetone. Nozzle: yellow, spraying level 4
<b>Bratton Marshall</b>	Solvent A: 0.1 g NaNO <sub>2</sub> in 1 mL H <sub>2</sub> O, add mixture of methanol and HCl (8.5 mL + 1.5 mL) to 10 mL. Solvent B: 0.1 g 4-(1-Naphtyl)-ethylendiamine-dihydrochloride in 1 mL H <sub>2</sub> O, add ethanol to 10 mL. Both solvents: blue nozzle, spraying level 4
<b>Chlor/o-Tolidin</b>	32 mg o-tolidine in 6 mL acetic acid and dilute with 100 mL of water. Add 0.20 g of KI (potassium iodide) and dissolve. Nozzle: Yellow; spraying level: 4
<b>Cobalt Thiocyanat Reagent</b>	1 mL of a 4 % cobalt thiocyanat solution with 1 mL of a 50 % glycerin solution and 1 mL H <sub>2</sub> O. Nozzle: red, spraying level 4
<b>Copper(II) sulfate Reagent</b>	10 % CuSO <sub>4</sub> in 8% o-phosphoric acid Nozzle: red, spraying level 5

Reagent	Preparation / Recipe / Remarks
<b>Copper(II) sulfate Reagent (Lipids)</b>	1.5 g CuSO <sub>4</sub> dissolved in small amount of water. Add methanol to 100 mL Nozzle: blue, spraying level 5
<b>Copper(II) sulfate Reagent (Ceramide)</b>	20 g CuSO <sub>4</sub> in 200 mL methanol. Add 8 mL sulfuric acid and 8 mL phosphoric acid 85 % Nozzle: red, spraying level 4
<b>Dichlorophenol indophenol reagent</b>	0.2 g/L in ethanol. Nozzle: yellow, spraying level 4
<b>2,4-Dinitrophenylhydrazin</b>	100 mg 2,4-dinitrophenylhydrazine in 90 mL ethanol and 10 mL concentrated hydrochloric acid (36 % HCl). Nozzle: Yellow; spraying level: 4
<b>L-DOPA in phosphate buffer (also in Triton / CHAPS)</b>	1 % PEG 8000: Nozzle: blue, spraying level 5 0.5% PEG 400: Nozzle: green, spraying level 6 1 % Triton: Nozzle: blue 0.1 % - 0.25 % CHAPS: Nozzle: blue / green, spraying level 6
<b>DPPH in methanol</b>	0.05 % DPPH (2,2-diphenyl-1-picrylhydrazyl) in methanol; clean chamber and holder thoroughly after spraying. Nozzle: blue, spraying level 2
<b>Fast Blue Salt (Echtblausalz B)</b>	50 mg Fast Blue Salt in 2 mL H <sub>2</sub> O, 5 mL methanol and 3 mL dichloromethane. Nozzle: blue, spraying level 3
<b>Glucose reagent</b>	100 mg glucose in 8 ml H <sub>2</sub> O and 0.5 mL H <sub>2</sub> SO <sub>4</sub> Nozzle: red, spraying level 4
<b>Glucosidase Inhibition Assay reagents</b>	12 mg 2-naphthyl-D-glucopyranoside in 9 mL ethanol and 1 mL 0.01 M NaCl solution Nozzle: yellow, spraying level 6  0.5 mL sodium acetate buffer (10.3 g sodium acetate in 250 mL water, adjusted to pH 7.5 with 0.1-M acetic acid) Nozzle: yellow, spraying level 6  Glucosidase solution (10 U/mL sodium acetate buffer, pH 7.5) Nozzle: yellow, spraying level 6  0.4 mL aqueous Fast Blue B salt solution (2 mg/mL) Nozzle: yellow, spraying level 6
<b>Glucuronidase Inhibition Assay reagents</b>	Phosphate buffer (pH7.0)  Glucuronidase solution (25 U/mL phosphate buffer plus 0.25 g BSA) X-Gluc substrate solution (2 mg/mL in water)  All reagents: Nozzle: red, spraying level 6
<b>Hanessians staining reagent</b>	2.5 g ammonium molybdate tetrahydrate ((NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> × 4 H <sub>2</sub> O) and 1.0 g ammonium cis (IV) sulfate dihydrate (Ce (NH <sub>4</sub> ) <sub>4</sub> (SO <sub>4</sub> ) <sub>4</sub> × 2 H <sub>2</sub> O) in 90 mL H <sub>2</sub> O or suspend. Add 10 mL H <sub>2</sub> SO <sub>4</sub> (concentrated sulfuric acid) and filter. Nozzle: Yellow; spraying level: 5
<b>Iodine</b>	50 mg iodine in 10 mL ethanol. Nozzle: blue / yellow, spraying level 4 / 6

Reagent	Preparation / Recipe / Remarks
<b>Iodine in hydrochloric acid</b>	5 mL iodine solution 0.05 mol/L, 1 mL sulfuric acid 37 % in 4 mL methanol Nozzle: yellow, spraying level 6
<b>Iodoplatinate reagent</b>	350 mg chloroplatinic acid hydrate in 70 mL 5% KI in H <sub>2</sub> O + 2 mL HCl Nozzle: blue
<b>Malachite green - ammonium molybdate spraying reagent</b>	Nozzle: yellow, spraying level 6
<b>Mandelin reagent Ammonium metavanadate</b>	Add dropwise 1 mL H <sub>2</sub> SO <sub>4</sub> in 9 ml methanol and dissolve 100 mg ammonium metavanadate. Nozzle: blue, spraying level 4
<b>Marquis reagent (methanol / sulfuric acid / formaldehyde)</b>	Add 8.5 mL methanol and 1.5 mL sulfuric acid. Allow the mixture to cool to room temperature, then add 0.5 mL formaldehyde. Nozzle: red / blue, spraying level 5
<b>N-(1-naphthyl)ethylenediamine (1%)</b>	100 mg N-(1-naphthyl)ethylenediamine dihydrochloride in 1 mL H <sub>2</sub> O + 9 mL ethanol Nozzle: blue / yellow
<b>Nitric acid</b>	20 % in water Nozzle: Yellow, spraying level 3
<b>Nitro blue tetrazolium chloride</b>	5 g/L in water Nozzle: red, spraying level 5
<b>Orcinol reagent</b>	20 mg orcinol in 40 mL acetone and 2mL H <sub>2</sub> SO <sub>4</sub> (conc). Nozzle: Yellow, spraying level 4
<b>Phloroglucinol reagent</b>	10 g/L in ethanol Nozzle: green, spraying level 3  0.1 g phloroglucinol in 1 mL ethanol and 9 mL HCl 37 % Nozzle: red, spraying level 6
<b>Phosphate buffer (for neutralization)</b>	0.75 mL phosphate buffer solution (8 g disodium hydrogen phosphate in 60 mL water adjusted to pH 7.5 – 7.8 with 0.1 M citric acid) Nozzle: yellow, spraying level 6  Phosphate-citrate buffer (8 % W/V, pH 7.8) Nozzle: green
<b>Potassium iodide</b>	0.12 g potassium iodide in 10 mL iodine (0.05 mL/L) and 10 mL methanol Nozzle: yellow, spraying level 4
<b>Potassium iodide-starch solution</b>	0.75 g of potassium iodide in 100 mL water. Boil for 2 min and add a suspension of 0.5 g of soluble starch in 35 mL of water. Boil for 2 min, cool to room temperature. Nozzle: yellow, spraying level 2 - 4
<b>Potassium permanganate, basic</b>	Dissolve 0.75 g KMnO <sub>4</sub> (potassium permanganate) and 5 g NaHCO <sub>3</sub> (sodium bicarbonate, sodium bicarbonate) in 100 mL H <sub>2</sub> O and add 0.6 mL 10 % NaOH (caustic soda). Nozzle: Yellow; spraying level: 3
<b>Primuline reagent</b>	20 mg of primuline in 200 mL acetone / water (80/20 v/v) Nozzle : blue, spraying level 3

Reagent	Preparation / Recipe / Remarks
<b>Resorcinol reagent</b>	20 mg of resorcinol dissolved in 1 mL water, add 8 mL HCl conc and 0.025 mL CuSO <sub>4</sub> (0.1 M). Dilute to 10 mL with water. Yellow nozzle, spraying level 4
<b>Sodium hydroxide reagent</b>	Sodium hydroxide 0.1 mol/L in water Nozzle: blue, spraying level 4
<b>Sodium periodate solution</b>	200 mg of NaIO <sub>3</sub> dissolved in 100 mL water. Nozzle: red, spraying level 3
<b>Tausky-Shorr reagent</b>	Tausky-Shorr-Stock solution, FeSO <sub>4</sub> in H <sub>2</sub> O Nozzle: yellow, spraying level 6.
<b>Tyrosinase in phosphate buffer</b>	400 U/mL Nozzle: blue / green, spraying level 5/6.
<b>van Urk reagent (4-(dimethylamino)-benzaldehyde in sulfuric acid)</b>	Under ice cooling, in a 10 mL volumetric flask, mix 4.5 mL of water with 1 mL of concentrated sulfuric acid. Allow the mixture to cool to room temperature and add 0.1 g of 4-(dimethylamino)-benzaldehyde. Fill up to 10 mL with water. Nozzle: red, spraying level 4.

## Problematic reagents and reagents that damage the device

This is not a comprehensive list of problematic substances: please consult chemical resistance charts to make certain that materials in direct contact with the reagents are resistant. For some specific combinations of reagents, micro-cracks may appear in the transparent hood with continued use. This does not affect the spraying and derivatization itself, but the service life of the hood is limited in such cases.

Derivatizer: Materials	
Nozzle housing	PET (standard nozzle) PEEK (Ultra nozzle)
Nozzle membrane	Palladium-alloy
Nozzle sealing ring	EPDM
Hood, transparent	PETG
Tray	PET
Tray seal	EPDM

Problematic Reagents / Reagents that damage the nozzle	Comment / Problem
Potassium hydroxide solution	Might be problematic if the standard nozzle (PET) is used: if cleaning is insufficient or nozzle is in direct contact over a longer period, the housing material becomes brittle. No problems with Ultra Nozzles (PEEK).
Iron(III) chloride (ferric chloride) solution in ethanol or in water	The metallic membrane is not resistant. This reagent will immediately damage the nozzle.
Potassium permanganate in sodium hydroxide solution	Due to the high surface-area-to-volume ratio of the aerosol, potassium hydroxide might react with atmospheric oxygen. This could cause a different color pattern compared to sprayed by hand.
Sulfuric acid solutions > 20 % in methanol ~ 10 % in ethanol	Due to the acid's high viscosity, spraying is not possible. Dilute with a suitable solvent (preferably with methanol) or replace part of ethanol with methanol to reduce viscosity.
Folin-Ciocalteu reagent (phosphomolybdate and phosphotungstate mixture)	Might corrodes metallic membrane
Peracetic acid (acetic peroxide)	Might corrodes metallic membrane
Antimony(III) chloride (in chloroform)	Salt precipitates in porous membrane