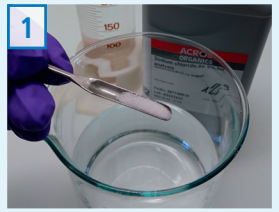
CAMAG Derivatizer

Recommendations for common derivatization reagents

Derivatization reagent	Use	Transfer from manual spraying 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	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



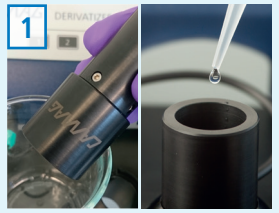
Dissolve **half of a spatula** (~ 300 mg) of sodium chloride (NaCl) in 500 mL water.

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

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

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

Cleaning of the nozzle



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

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

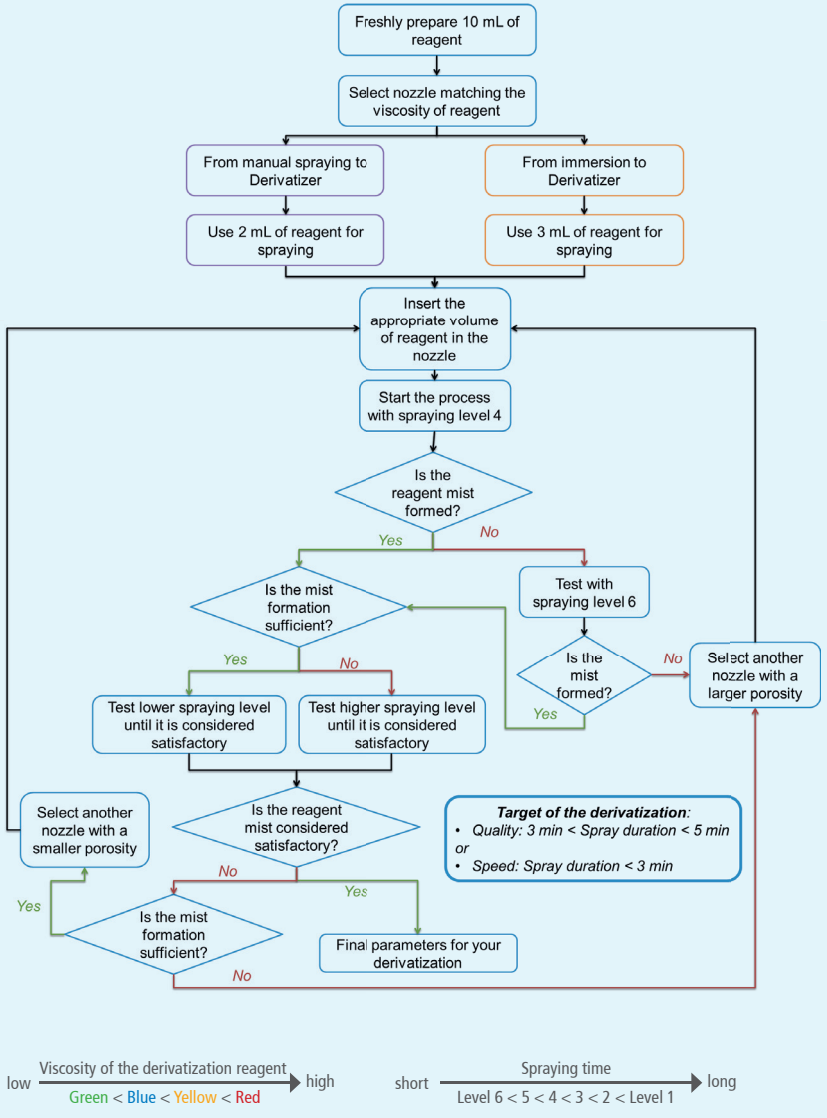
When necessary or when changing spraying reagent: **rinse the tip of the nozzle** and the inside of the reagent container with (tap) water, ethanol or cleaning solution.

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



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.

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 10 g of phosphomolybdic acid hydrate in 50 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 µL 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.

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