Vaast® DERIVATIZATION PROTOCOL

Chemicals Needed

Vaast®, REAGENT KIT AMINE DERIVATIZATION (Liquid), P/N: 72K01 or

Vaast®, COLUMN KIT, (72U93+72K01), P/N: 72KC1 composed of:

- **Vial A**: 6-Aminoquinolyl-N-hydroxysuccinimidyl carbamate / AQC derivatization reagent
- **Vial B**: Acetonitrile
- **Vial C**: Borate buffer reagent (in powder form)

Cysteine[1]-derivatization kit composed by:

- **Vial D**: Dithiothreitol
- **Vial E**: Iodoacetamide

Chemicals not included:

- 0.1 M Hydrochloric acid aqueous solution
- Purified water
- Acetonitrile HPLC grade if using:
  - **Vaast®, REAGENT KIT AMINE DERIVATIZATION (Powder), P/N: 72K02** or
  - **Vaast®, COLUMN KIT, (72U93+72K02), P/N: 72KC2**

Storage and Stability

The derivatization kit may be stored at room temperature before opening. For extended storage, it is advised keeping it at 4°C.

Reagents' expiry dates are indicated on their vial labels and are based on before opening or reconstitution.

Under no circumstances should the kits be used over expiry date.

Once reconstituted, the reagents and buffer solutions should be stored at room temperature in a desiccator with dry desiccant, and used within one week.

Derivatized amino acid standard solutions may be stored at room temperature for up to one week.

[1] *Available soon*

Protocol with Vaast® Derivatization Kits[2]

[2]Step 1: Preparation of AQC and Borate Buffer reagents *(common for all amino acids)*

Solution of AQC reagent (Vial A)

- Agitate vial A to ensure all powder is at bottom of vial.
- Transfer 1 mL of **Acetonitrile** from vial B to vial A and vortex for 10 sec.

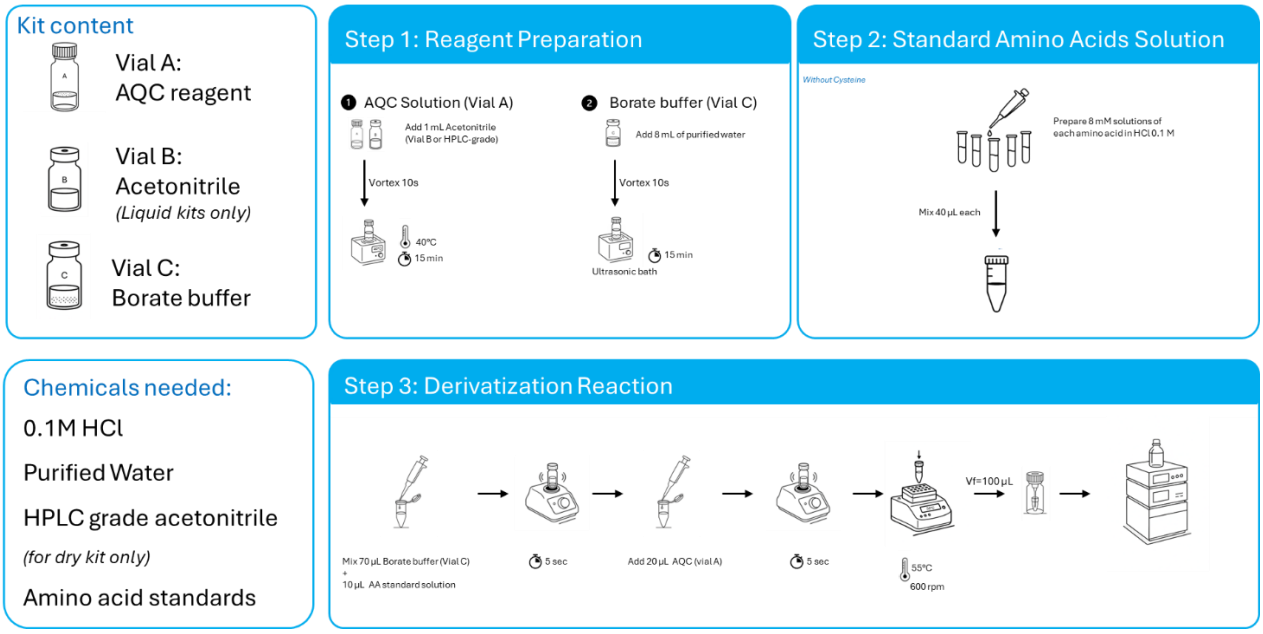
*(If **Vaast®, REAGENT KIT AMINE DERIVATIZATION (Powder)** or **Vaast®, COLUMN KIT, (72U93+72K02)** are used, add 1 mL of HPLC-grade Acetonitrile as acetonitrile is not supplied).*

- Heat in an ultrasonic bath at 40°C for 15 min until fully dissolved.

Reconstitution of Borate Buffer in solution (Vial C)

- Agitate vial C to ensure all powder is at bottom of vial.
- Add 8 mL of purified water and vortex for 10 sec.
- Place in ultrasonic bath for 15 min until fully dissolved.

General derivatization protocol of amino acids (<u>excluding Cysteine</u>):



Step 2 : Preparation of Amino acid standard solution(s)

- If Cysteine should be tested, please follow the dedicated protocol as described below.
- Prepare a solution of each Amino acid to be analyzed at 8 mM in HCl 0.1 M. (e.g., for Ala: 0.7mg in 1 mL).
- Prepare the **final Amino acid standard solution** by mixing 40 µL of each amino acid solution (final composition of the standard solution would be adapted to the purpose of the analysis). *Therefore, amino acids will be quantified in their AQC-form, except for Lys present in its bis-AQC-Lys form.*

Step 3 : Derivatization reaction

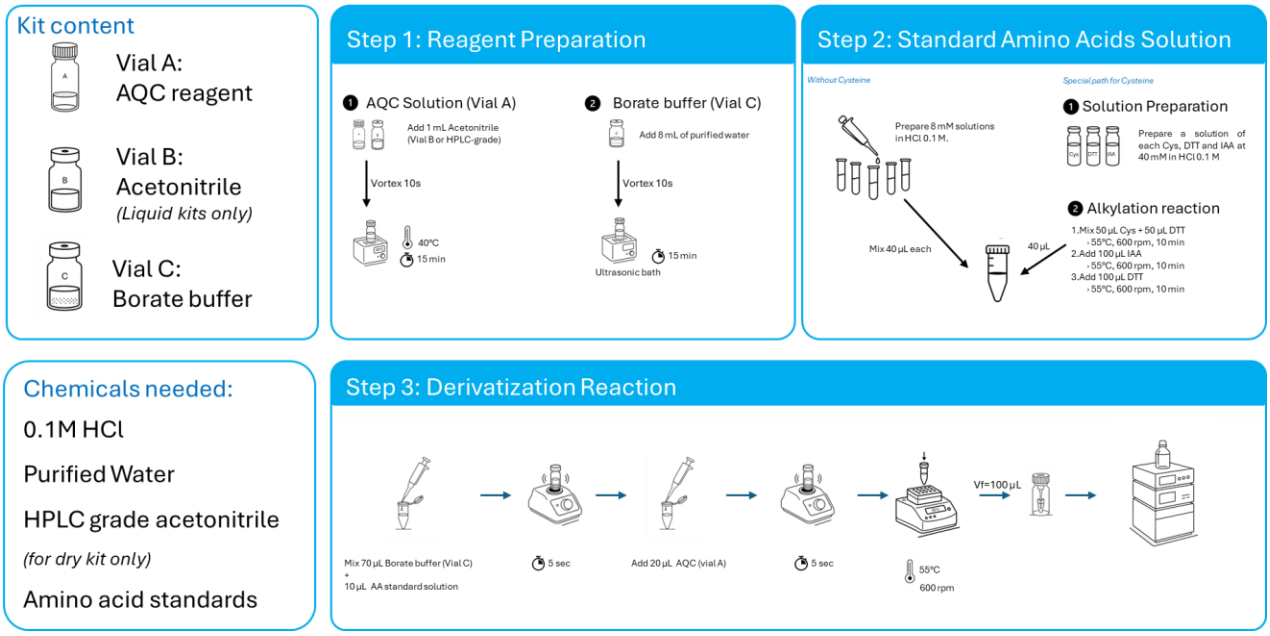
- Draw 70 µL of reconstituted **borate buffer solution** from vial C and transfer to a 100 µL Eppendorf.
- Add 10 µL of the final **Amino acid standard solution** and vortex for 5 sec.
- Add 20 µL of **AQC solution** from vial A to the Eppendorf and vortex for 5 sec.
- Heat on top of heating block at 55°C, 600 rpm,10 min.

[2] Protocol designed with a minimum 4-fold molar excess of AQC reagent versus amino acid to ensure full derivatization. If differently concentrated samples should be analysed, reagent concentrations should be adapted. Further applications with different samples to be available soon.

- Transfer to an HPLC vial fitted with a 100 µL insert.
- Sample is ready for analysis (global amino acids concentration 800 pmoL/µL).

Specific derivatization protocol of amino acids (<u>Cysteine included</u>):

Due to the propensity of cysteine to oxidize to cystine, samples containing cysteine should be pretreated through a short reduction–alkylation sequence (DTT → IAA → DTT) to stabilize free thiol groups and prevent disulfide formation. Therefore, Cys will be quantified in its AQC-Cys-IAA form.



Step 2 : if Cysteine is tested

- **Preparation of solutions:**
  - Prepare a Cys solution at 40 mM in HCl 0.1 M (That is, 4.85mg in 1 mL).
  - Prepare a solution of each remaining Amino acid to be analysed at 8 mM in HCl 0.1 M (e.g., for Ala: 0.7mg in 1 mL).
  - By sampling from **vial D** and **vial E**, prepare a solution of **DTT** at 40 mM in HCl 0.1 M (1.23 mg in 200 µL) and a solution of **IAA** at 40 mM in HCl 0.1 M (1.47 mg in 200 µL).

- **Alkylation Reaction:**
  - In an Eppendorf, mix 50 µL of **cysteine solution** and 50 µL of **DTT solution** and heat on top of heating block at 55°C and 600 rpm for 10 min.
  - Add 100 µL of **IAA solution** and heat on top of heating block at 55°C and 600 rpm for 10 min.
  - Add 50 µL of **DTT solution** and heat on top of heating block at 55°C and 600 rpm for 10 min.

Prepare the **final Amino acid standard solution** by mixing 40 µL of each amino acid solution, including the one of alkylated Cys (final composition of the standard solution would be adapted to on the purpose of the analysis).

Step 3 : Derivatization reaction

- Draw 70 µL of reconstituted **borate buffer solution** from vial C and transfer to a 100 µL Eppendorf.
- Add 10 µL of the final **Amino acid standard solution** and vortex for 5 sec.
- Add 20 µL of **AQC solution** from vial A to the Eppendorf and vortex for 5 sec.
- Heat on top of heating block at 55°C, 600 rpm,10 min.
- Transfer to an HPLC vial fitted with a 100 µL insert.
- Sample is ready for analysis (global amino acids concentration 800 pmol/µL).

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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