

Cat.No. :D2K4101926

Amino Acid-AQC kit for determination of amino acids

Description:

6-aminoquinolyl-N-hydroxysuccinimidyl carbamate is a new fluorescent reagent for derivatization of amino acids. The reaction at pH 8,2-10 is very fast and quantitative, the derivatives are very stable. Selective fluorescence of derivatives enables direct injection of reaction product without the necessity of excess reagent removal.

Recommended derivatization procedure

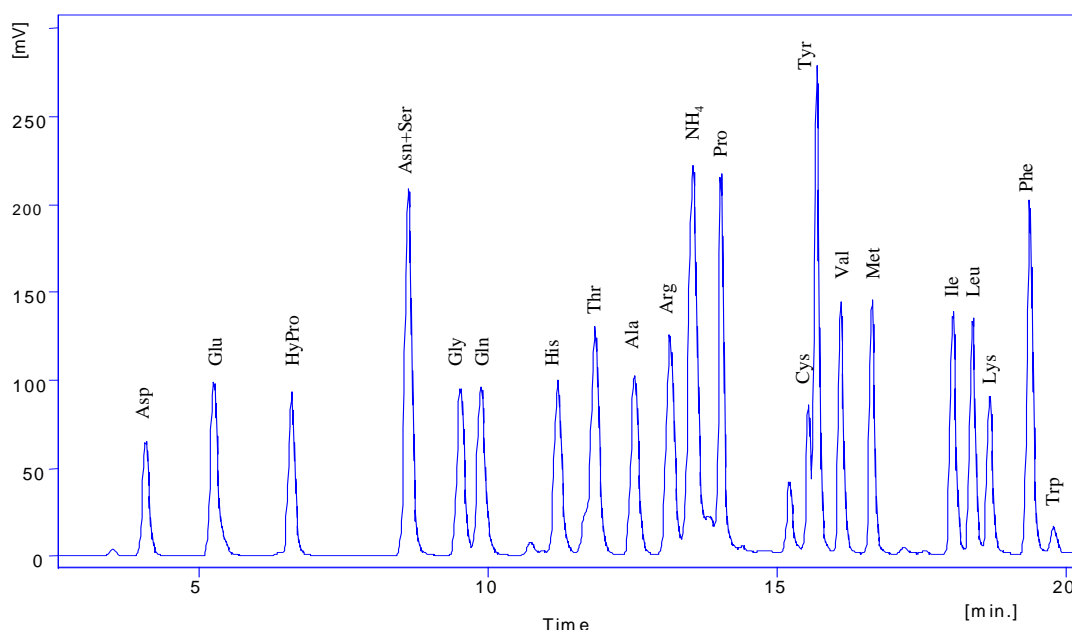
Mix 70 μ l of 0,2 M borate buffer pH 8,8, and 10 μ l sample Add 20 μ l of derivatizing reagent, and stir thoroughly.

Derivatized sample can be injected directly or diluted with eluent.

Detection: fluorimetric (excitation 254 nm; emission 395 nm) or UV (254 nm).

Total concentration of aminogroups in sample should not exceed 4 mM.

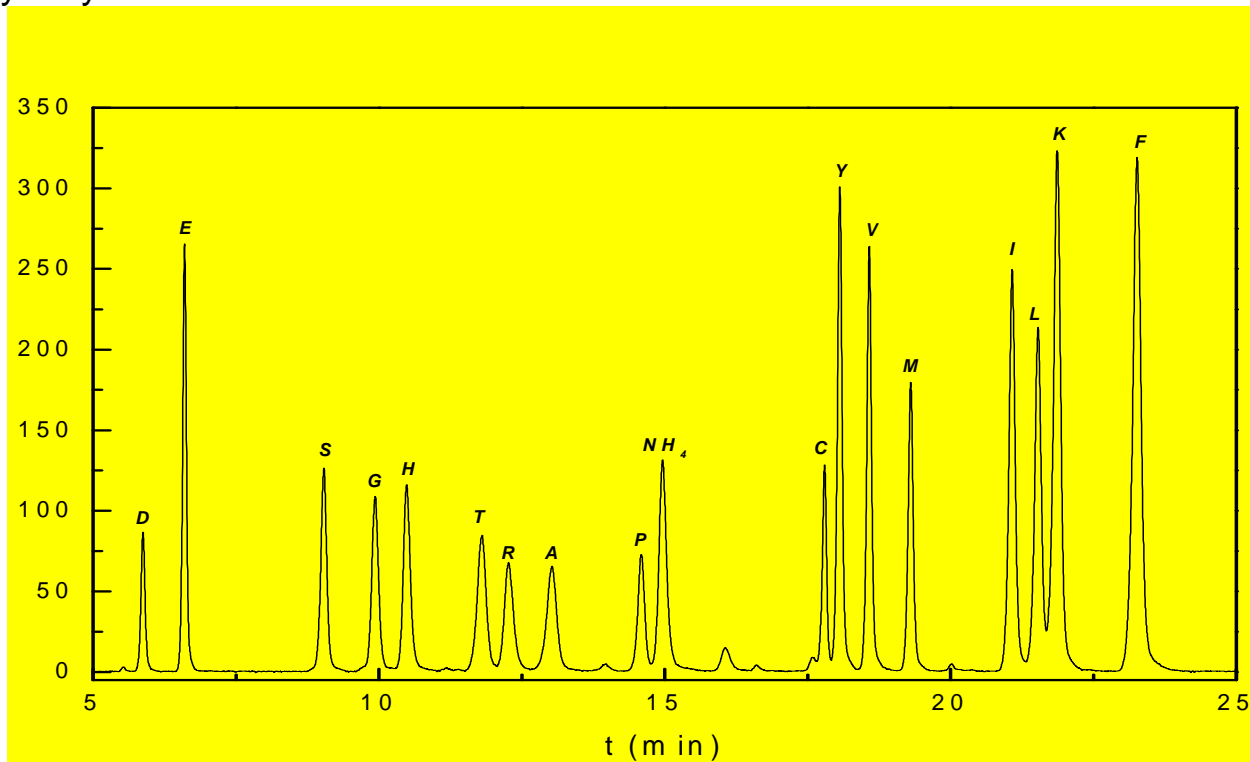
Separation of AQC amino acids – AA-standard



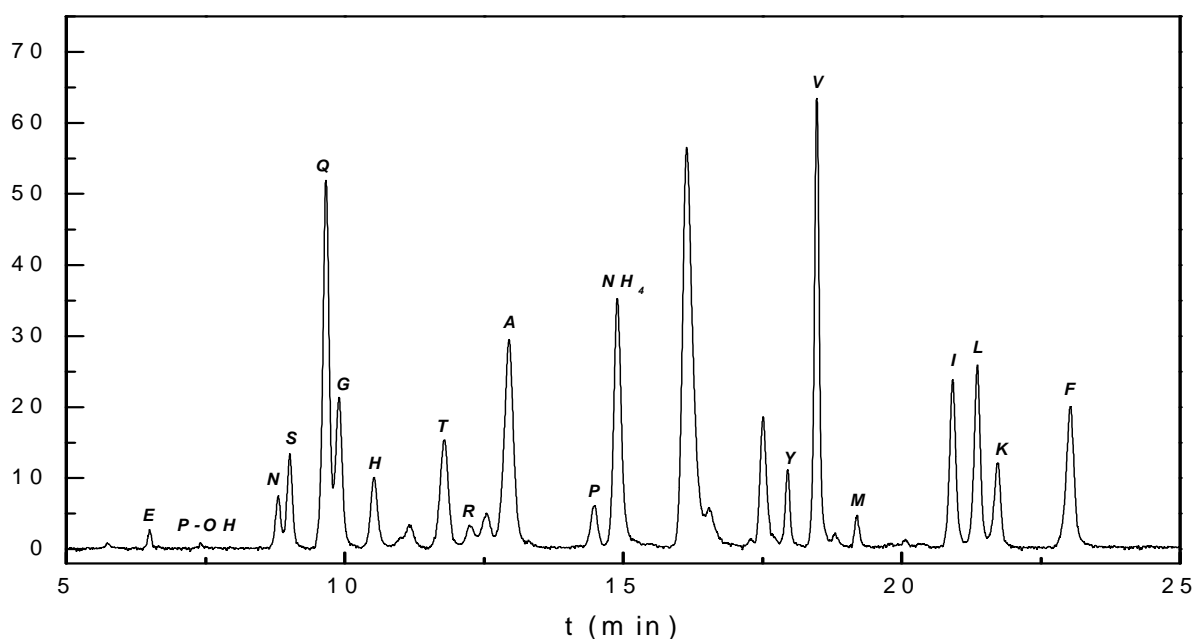
Column: WATREX 250x4 mm Amino Acid-AQC 5 μ m with 20x4mm guard column
Eluent A: 0,1M acetate buffer pH 5,0 (adjusted with H₃PO₄)
Eluent B: Acetonitrile-water 60:40
Flow rate: 2 ml/min
Temperature: 37°C
Gradient: 0 min 5%B; 11,5 min 16%B; 11,75 min 25%B; 15 min 30%B; 17,5 min 35%B; 21 min 50%B; 22 min 100%B; 29 min 100%B; 30 min 5%B; 37 min 5%B
Pressure: 20-24 MPa

Applications for Amino Acid - AQC kit

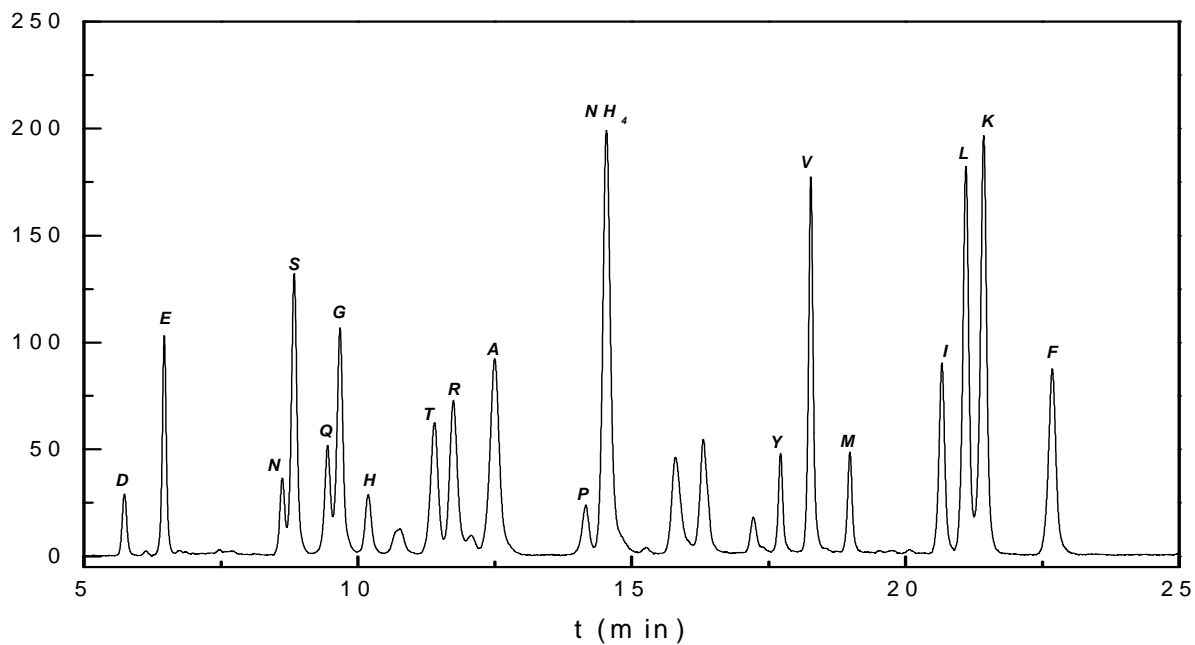
Hydrolysate standard



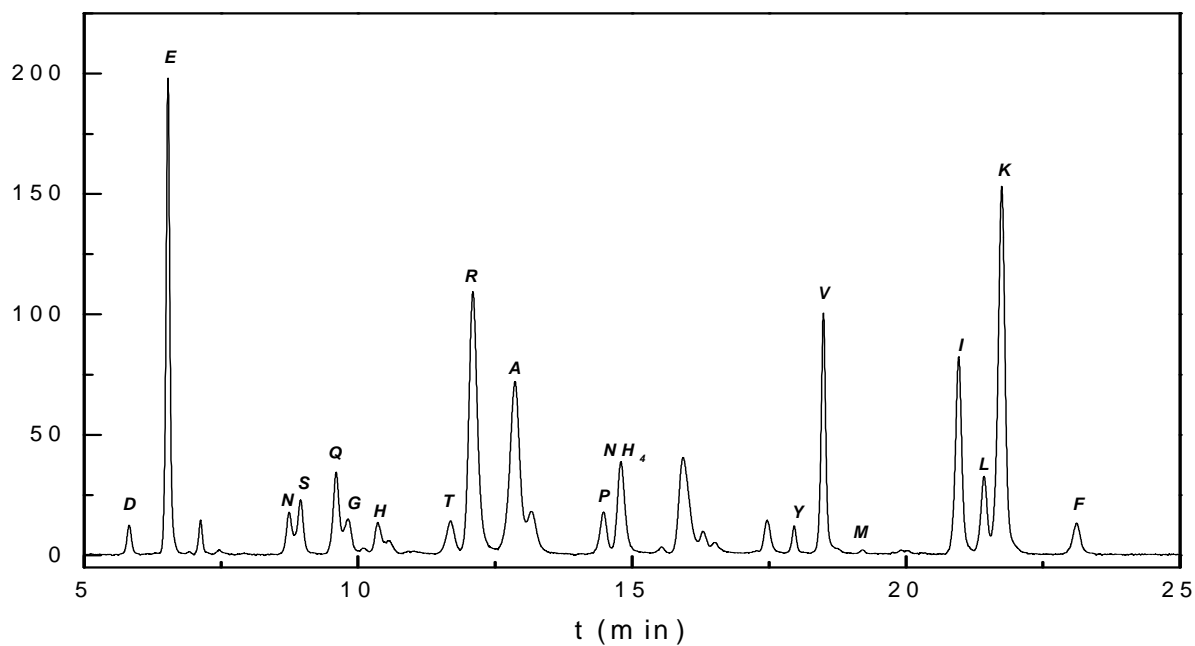
Human plasma (free AA)

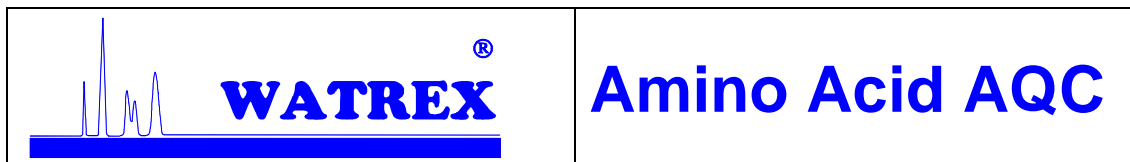


Colon tissue biopsy extract (free AA)



Yeast cells (free AA)





HPLC conditions for above applications:

Column:	WATREX 250x4 mm Amino Acid-AQC 5 um with 20x4mm guard column
Eluent A:	0,1M acetate buffer pH 6,4 (adjusted with H ₃ PO ₄)
Eluent B:	Acetonitrile-water 60:40
Flow rate	1,5 ml/min
Temperature:	37°C
Gradient:	0 min 5%B; 4 min 18%B; 10 min 19%B; 16 min 35%B; 24 min 40%B; 25 min 60%B; 31 min 60%B; 32 min 5%B; 39 min 5%B
Pressure:	20-24 MPa

Amino acid isolation

Plasma

100 µl plasma was added to 400 µl acetonitrile, vortexed and centrifuged for 10 min at 10 000 x g.

Colon tissue

10-30 mg colon tissue was incubated in 200 µl water for 10 min at 50°C. Mixture was briefly centrifugated and 100 ul supernatant was added to 400 µl acetonitrile, vortexed and centrifuged for 10 min at 10 000 x g

Yeast cells

Two or three yeast colonies were suspended in 3 ml of water and amino acids were extracted following the method of Ohsumi et al. (1988) modified by Gent and Slaughter (1998).

Reference

Analytical Biochemistry 211, 279-287 (1993)

Gent, D.P., and Slaughter, J.C. (1998). Intracellular distribution of amino acids in an slp1 vacuole-deficient mutant of the yeast *Saccha romyces cerevisiae*. J, Appl. Microbiol. 84, 752-758

Ohsumi, Y., Kitamoto, K., and Anraku, Y. (1988). Changes induced in the permeability barrier of the yeast plasma membrane by cupric ion, J. Bacteriol. 170, 2676-2782

Amino Acid-AQC contains:

D2K4101927	5x1 ml derivatizing reagent AQC for determination of amino acids (solution of 6-aminochinolyl-N-hydroxysuccinimidyl carbamate in acetonitrile)
D2K4101928	HPLC column WATREX 250x4 mm Amino Acid-AQC 5 um
D2K4101929	Set of 3 pcs guard column WATREX 20x4 mm Amino Acid-AQC 5 um
WI029012	WATREX integral guard column holder Instructions for derivatization and separation conditions