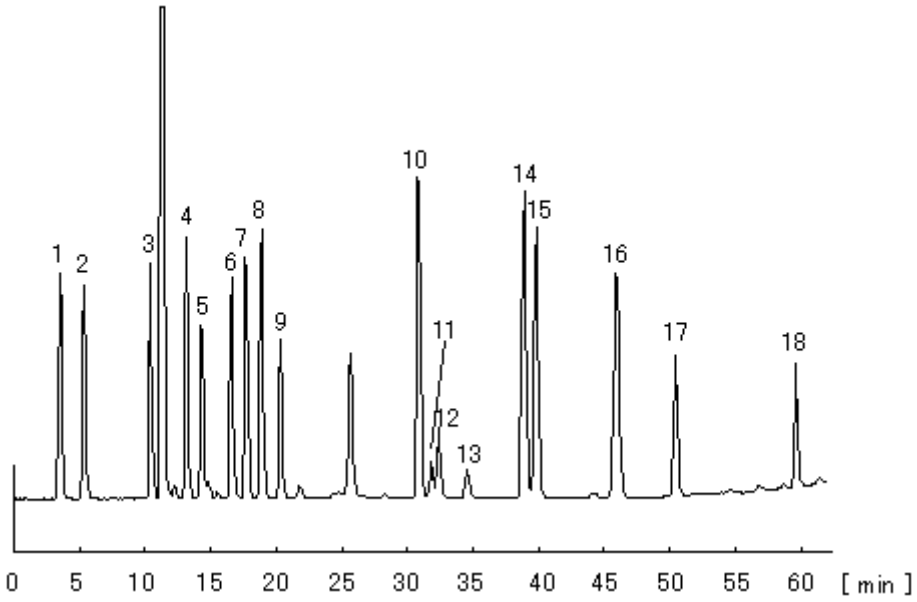
NBD-**アミノ酸** NBD-Amino acids

蛍光誘導体化試薬 NBD-F(4-Fluoro-7-nitrobenzofurazan)**は，第一・第二アミノ酸などと温和な条件で極めて反応性に富み，発蛍光体は安定です．励起，蛍光波長が比較的長波長の強い蛍光を発します．試薬自身は蛍光を持ちません．** HPLC **におけるアミン・アミノ酸の蛍光ラベル化剤として適しています．**

4-Fluoro-7-nitrobenzofurazan (NBD-F), a fluorescence derivatizing reagent, reacts with primary and secondary amines under mild conditions, and generates stable derivatives with relatively long emission and excitation wavelengths. The reagent does not fluoresce itself.



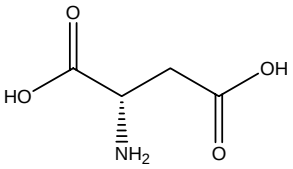
1. L-**アスパラギン酸** (0.6 pmol)
   L-Aspartic acid (M.W. 133.1)
2. L-**グルタミン酸** (0.6 pmol)
   L-Glutamic acid (M.W. 147.1)
3. L-**セリン** (0.6 pmol)
   L-Serine (M.W. 105.9)
4. **グリシン** (0.6 pmol)
   Glycine (M.W. 75.1)
5. L-**ヒスチジン** (0.6 pmol)
   L-Histidine (M.W. 155.2)
6. L-**スレオニン** (0.6 pmol)
   L-Threonine (M.W. 119.1)
7. L-**アラニン** (0.6 pmol)
   L-Alanine (M.W. 89.1)
8. L-**アルギニン** (0.6 pmol)
   L-Arginine (M.W. 174.2)
9. L-**プロリン** (0.6 pmol)
   L-Proline (M.W. 115.1)
10. L-**バリン** (0.6 pmol)
    L-Valine (M.W. 117.2)
11. L-**シスチン** (0.6 pmol)
    L-Cystine (M.W. 240.3)
12. L-**メチオニン** (0.6 pmol)
    L-Methionine (M.W. 149.1)
13. **塩化アンモニウム** (0.6 pmol)
    Ammonium chloride (M.W. 53.5)
14. L-**ロイシン** (0.6 pmol)
    L-Leucine (M.W. 131.2)
15. L-**イソロイシン** (0.6 pmol)
    L-Isoleucine (M.W. 131.2)
16. L-**フェニルアラニン** (0.6 pmol)
    L-Phenylalanine (M.W. 165.2)
17. L-**リジン** (0.6 pmol)
    L-Lysine (M.W. 146.2)
18. L-**チロシン** (0.6 pmol)
    L-Tyrosine (M.W. 181.2)

【HPLC Conditions】

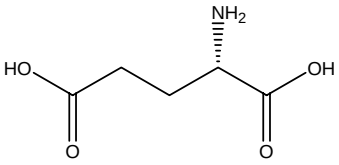
Column	:	CAPCELL PAK C_{18} MG S5；1.5 mm i.d. x 150 mm
Mobile phase	:	A) Sodium perchlorate was added to 10 mmol/L citrate buffer (0.08 g citric acid monohydrate and 1.36 g Trisodium citrate dihydrate were dissolved in 500 mL H_2O) at 75 mmol/L。 B) CH_3CN / H_2O = 50 / 50 B 5 % -> 37 % (40.0 min) -> 38 % (52.0 min) -> 100 % (60.0 min) -> 100 % (65.0 min) -> 5 % (65.1 min) -> 5 % (80.0 min) Gradient
Flow rate	:	100 μL/min
Temperature	:	40 ˚C
Detection	:	FL Ex. 480nm, Em. 530nm
Inj. vol.	:	1 μL
Pretreatment	:	Standard amino acids solution Type H（17 compounds, 2.5 μmol/mL each, in 0.1mol/L HCl, Wako Chemicals, Osaka, Japan and other five amino acids (0.1 mol/L HCl solution) were mixed together The mixture was diluted to 0.5 μmol/L with 0.1 mol/L borate buffer (0.28 g of boric acid and 2.0 g of sodium tetraborate decahydrate were dissolved in 100 mL of water). And then, NBD-Fderivatization was carried out on the solution. (For the detailed derivatization protocol, refer to Technical Report “NBD- Amino Acids”.)

【References】

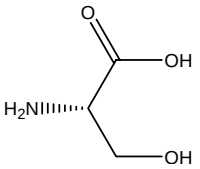
和光純薬工業株式会社 (Wako LIFE SCIENCE '95/'96 3RD EDITION)



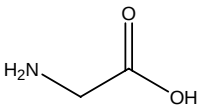
1. L-Aspartic acid



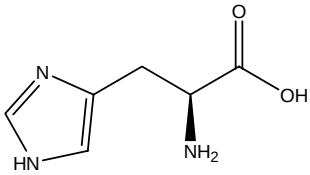
2. L-Glutamic acid



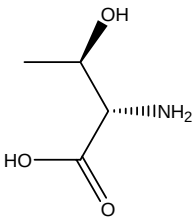
3. L-Serine



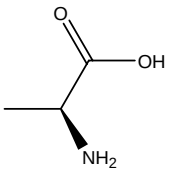
4. Glycine



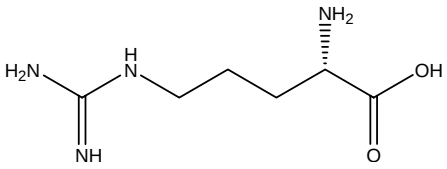
5. L-Histidine



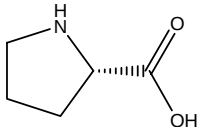
6. L-Threonine



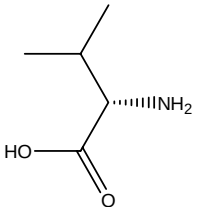
7. L-Alanine



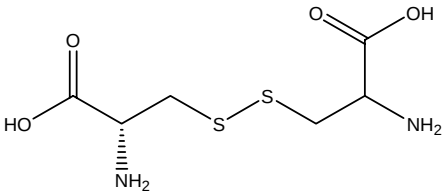
8. L-Arginine



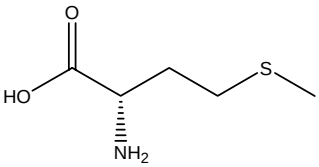
9. L-Proline



10. L-Valine



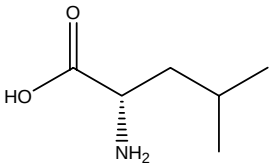
11. L-Cystine



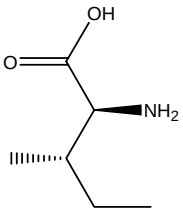
12. L-Methionine

$NH_4^+ \quad Cl^-$

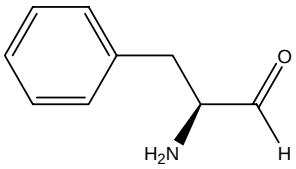
13. Ammonium chloride



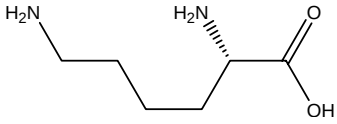
14. L-Leucine



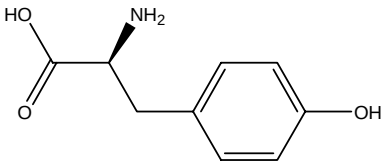
15. L-Isoleucine



16. L-Phenylalanine



17. L-Lysine



18. L-Tyrosine