

# Chromatography

## NUCLEODUR<sup>®</sup> 300

HPLC columns for  
biochromatography



**MACHERY-NAGEL**

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*Since 1911*

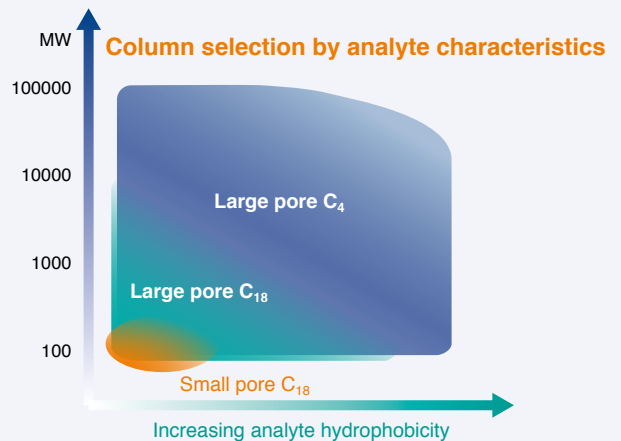
## NUCLEODUR® 300 C<sub>4</sub>/C<sub>18</sub>

- **Key features:**
  - Reliable widepore RP phases for daily routine analysis
  - Medium density octadecyl or butyl modification with exhaustive endcapping
  - Ideal phases for separation of biomolecules
- **Technical characteristics:**

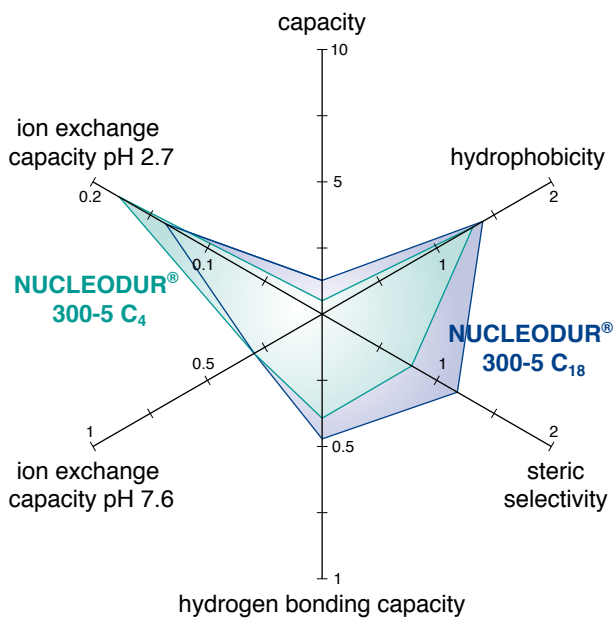
Pore size 300 Å; particle size 5 µm;  
carbon content 4% for C<sub>18</sub>, 2.5% for C<sub>4</sub>;  
pH stability 1–9, high reproducibility from lot to lot
- **Recommended applications:**

Biological macromolecules like proteins or peptides  
**USP L1 (C<sub>18</sub>) / USP L26 (C<sub>4</sub>)**

## wide pore silica for biochromatography



## Tanaka plots of NUCLEODUR® wide pore phases



## Stability test of NUCLEODUR® 300-5 C<sub>4</sub> ec

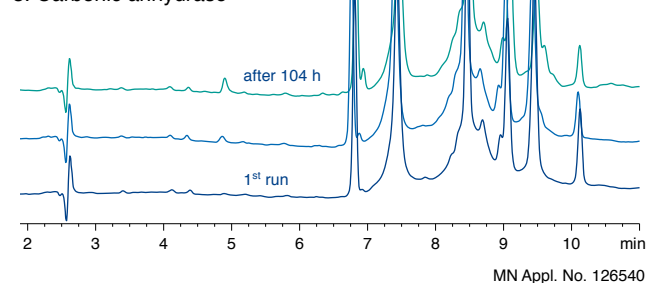
Column: 250 x 4.6 mm NUCLEODUR® 300-5 C<sub>4</sub> ec

Eluent: A) 0.1 % TFA in water  
B) 0.08 % TFA in acetonitrile  
20–50 % B in 11 min

Flow rate: 1.3 mL/min  
Temperature: 25 °C  
Detection: UV, 280 nm

### Peaks:

1. Insulin
2. Trypsinogen
3. Lactalbumin
4. Myoglobin
5. Carbonic anhydrase



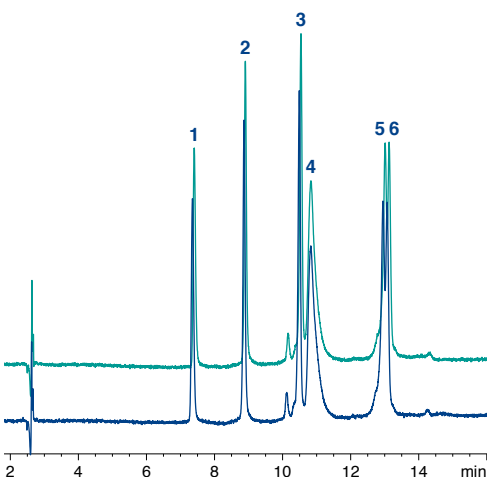
## Batch-to-batch reproducibility of NUCLEODUR® C<sub>4</sub> ec and NUCLEODUR® C<sub>18</sub> ec wide pore phases

Columns: 250 x 4 mm  
Eluent: A) 0.1 % TFA in water  
B) 0.08 % TFA in acetonitrile  
20–60 % B in 15 min  
Flow rate: 1 mL/min  
Temperature: 25 °C  
Detection: UV, 280 nm

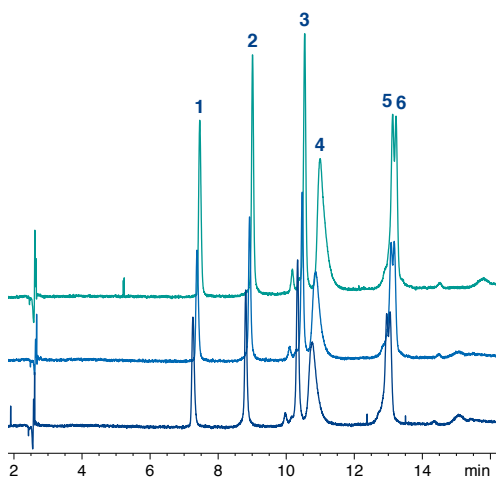
### Peaks:

1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. β-Lactoglobulin
6. β-Lactoglobulin 2

### NUCLEODUR® 300-5 C<sub>4</sub> ec



### NUCLEODUR® 300-5 C<sub>18</sub> ec

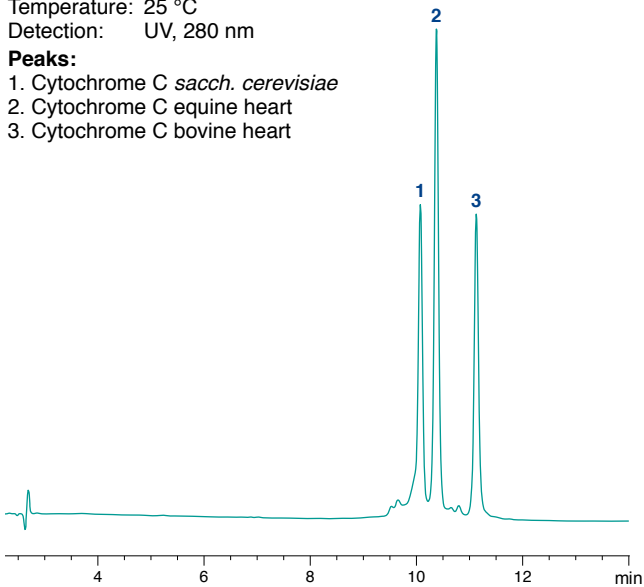


MN Appl. No. 126551/126552

### Cytochrome C test

Column: **250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec**  
 Eluent: A) 0.1 % TFA in water  
 B) 0.08 % TFA in acetonitrile  
 20–60 % B in 15 min (2 min 60 % B)  
 Flow rate: 1.3 mL/min  
 Temperature: 25 °C  
 Detection: UV, 280 nm

- Peaks:**  
 1. Cytochrome C *sacch. cerevisiae*  
 2. Cytochrome C equine heart  
 3. Cytochrome C bovine heart



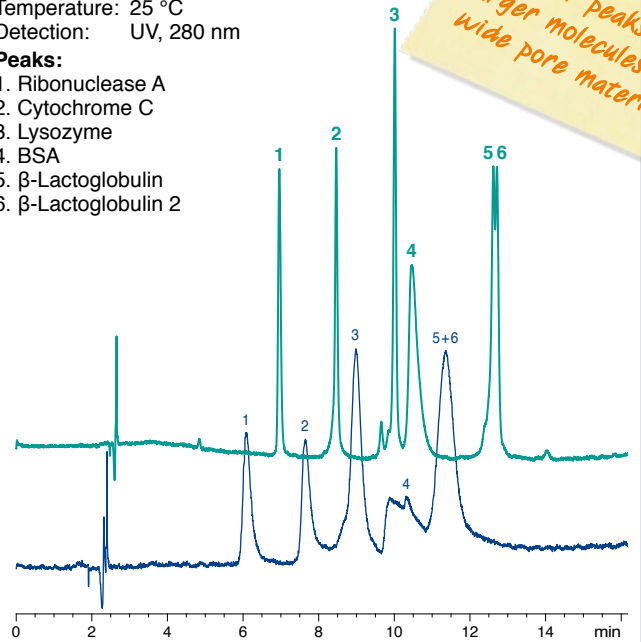
MN Appl. No. 126560

### Comparison of narrow and wide pore NUCLEODUR® for the separation of proteins

Columns: **250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec**  
**250 x 4.6 mm NUCLEODUR® C<sub>18</sub> Gravity, 5 µm**  
 Eluent: A) 0.1 % TFA in water  
 B) 0.08 % TFA in acetonitrile  
 20–65 % B in 15 min  
 (3 min 65 % B)

Flow rate: 1.3 mL/min  
 Temperature: 25 °C  
 Detection: UV, 280 nm

- Peaks:**  
 1. Ribonuclease A  
 2. Cytochrome C  
 3. Lysozyme  
 4. BSA  
 5. β-Lactoglobulin  
 6. β-Lactoglobulin 2



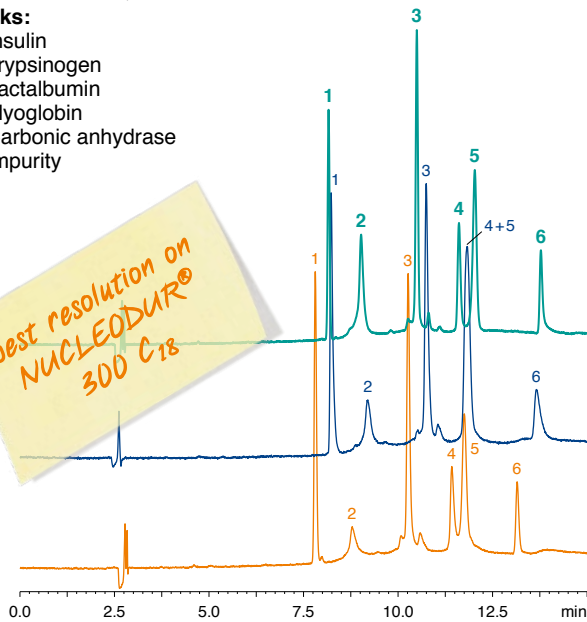
MN Appl. No. 126590

### Column comparison for protein separation

Columns: **250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec**  
**250 x 4.6 mm NUCLEOSIL® 300-5 C<sub>18</sub>**  
**250 x 4.6 mm Jupiter® C<sub>18</sub>, 5 µm**

Eluent: A) 0.1 % TFA in water  
 B) 0.08 % TFA in acetonitrile  
 20–65 % B in 15 min (2 min 65 % B)  
 Flow rate: 1.3 mL/min  
 Temperature: 25 °C  
 Detection: UV, 280 nm

- Peaks:**  
 1. Insulin  
 2. Trypsinogen  
 3. Lactalbumin  
 4. Myoglobin  
 5. Carbonic anhydrase  
 6. Impurity



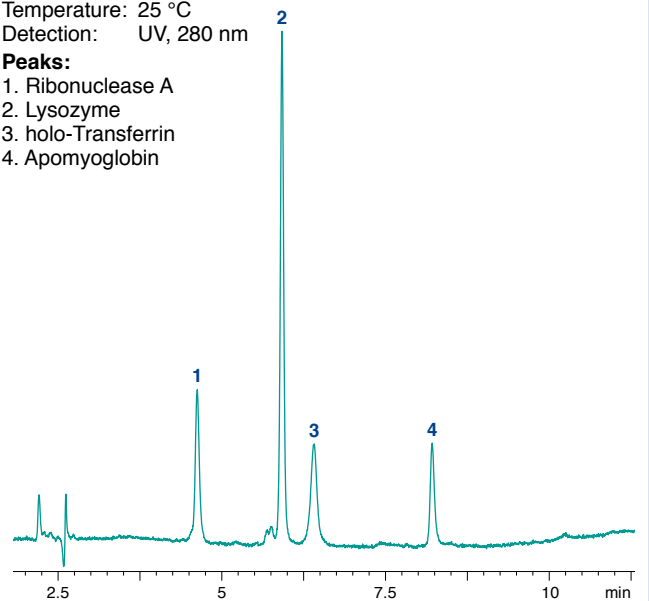
MN Appl. No. 126570

### Protein test

Column: **250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec**  
 Eluent: A) 0.1 % TFA in water  
 B) 0.08 % TFA in acetonitrile  
 25–60 % B in 8.5 min (1.5 min 60 % B)

Flow rate: 1.3 mL/min  
 Temperature: 25 °C  
 Detection: UV, 280 nm

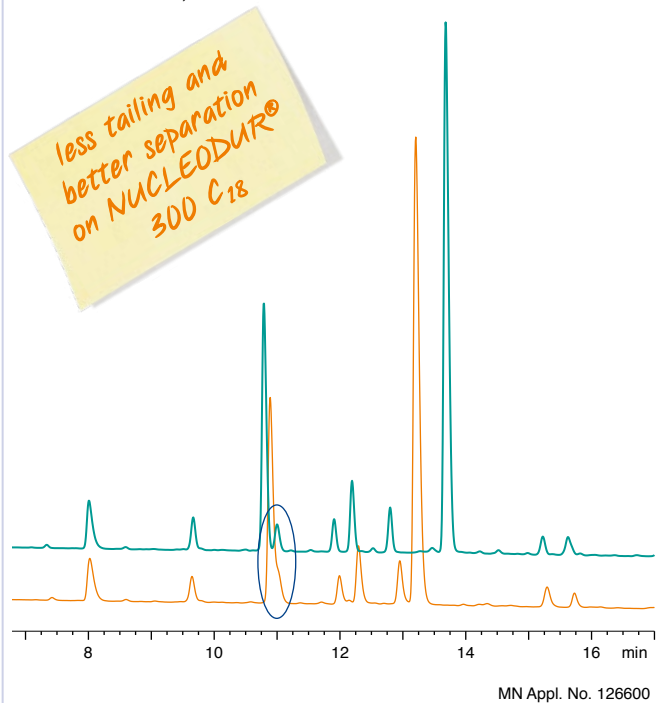
- Peaks:**  
 1. Ribonuclease A  
 2. Lysozyme  
 3. holo-Transferrin  
 4. Apomyoglobin



MN Appl. No. 126580

### Tryptic digest of cytochrome C

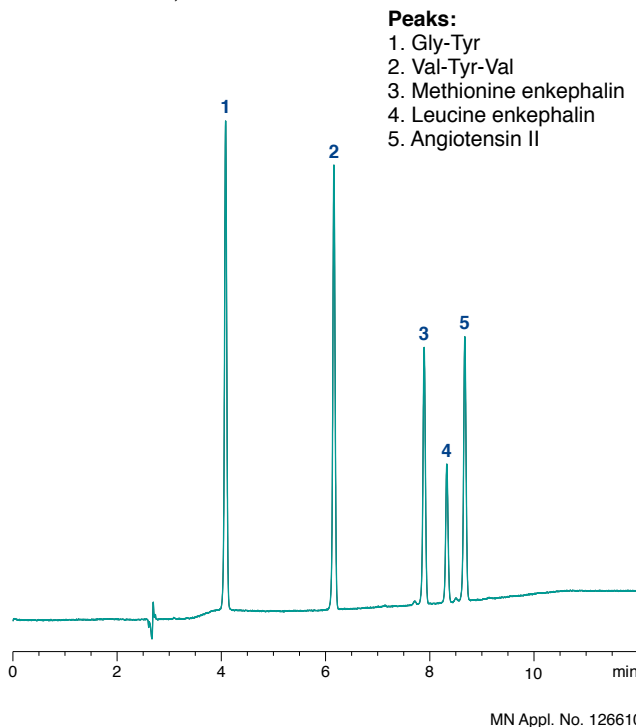
Columns: 250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec  
 250 x 4.6 mm Jupiter® C<sub>18</sub>, 5 µm  
 Eluent: A) 0.1% TFA in water  
 B) 0.08% TFA in acetonitrile  
 5–40% B in 15 min (1 min 40% B)  
 Flow rate: 1.3 mL/min  
 Temperature: 30 °C  
 Detection: UV, 280 nm



MN Appl. No. 126600

### Peptide test

Columns: 250 x 4.6 mm NUCLEODUR® 300-5 C<sub>18</sub> ec  
 Eluent: A) 0.1% TFA in water  
 B) 0.08% TFA in acetonitrile  
 10–40% B in 7.5 min (2 min 40% B)  
 Flow rate: 1.3 mL/min  
 Temperature: 25 °C  
 Detection: UV, 280 nm



**Peaks:**  
 1. Gly-Tyr  
 2. Val-Tyr-Val  
 3. Methionine enkephalin  
 4. Leucine enkephalin  
 5. Angiotensin II

MN Appl. No. 126610

## Ordering information · EC columns

### NUCLEODUR® 300-5 C<sub>18</sub> ec

Octadecyl modification, endcapped, pore size 300 Å, particle size 5 µm, eluent in column acetonitrile – water

Length →	100 mm	125 mm	150 mm	250 mm	EC guard columns*	CC guard columns**
2 mm ID	760183.20	760184.20	760185.20	760186.20	761988.20	761008.30
3 mm ID	760183.30	760184.30	760185.30	760186.30	761988.30	761008.30
4 mm ID	760183.40	760184.40	760185.40	760186.40	761988.30	761008.40
4.6 mm ID	760183.46	760184.46	760185.46	760186.46	761988.30	761008.40

### NUCLEODUR® 300-5 C<sub>4</sub> ec

Butyl modification, endcapped, pore size 300 Å, particle size 5 µm, eluent in column acetonitrile – water

Length →	100 mm	125 mm	150 mm	250 mm	EC guard columns*	CC guard columns**
2 mm ID	760193.20	760194.20	760195.20	760196.20	761989.20	761009.30
3 mm ID	760193.30	760194.30	760195.30	760196.30	761989.30	761009.30
4 mm ID	760193.40	760194.40	760195.40	760196.40	761989.30	761009.40
4.6 mm ID	760193.46	760194.46	760195.46	760196.46	761989.30	761009.40

\* EC guard columns require the Column Protection System guard column holder 718966

\*\* CC guard columns are connected using the ChromCart® guard column holder 721359

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