

Chromatography

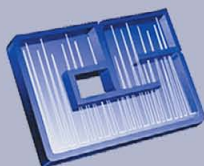
NUCLEODUR® 300

HPLC columns for
biochromatography



MACHERY-NAGEL

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**CHROMATOGRAPHIC
SPECIALTIES INC.**

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NUCLEODUR® 300 C₄/C₁₈

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:

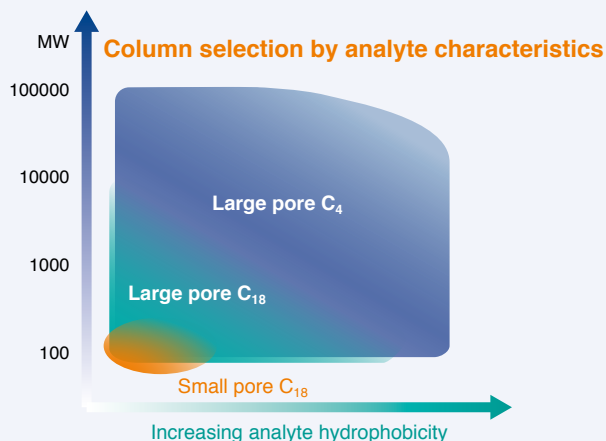
Pore size 300 Å; particle size 5 µm;
carbon content 4 % for C₁₈, 2.5 % for C₄;
pH stability 1–9, high reproducibility from lot to lot

Recommended applications:

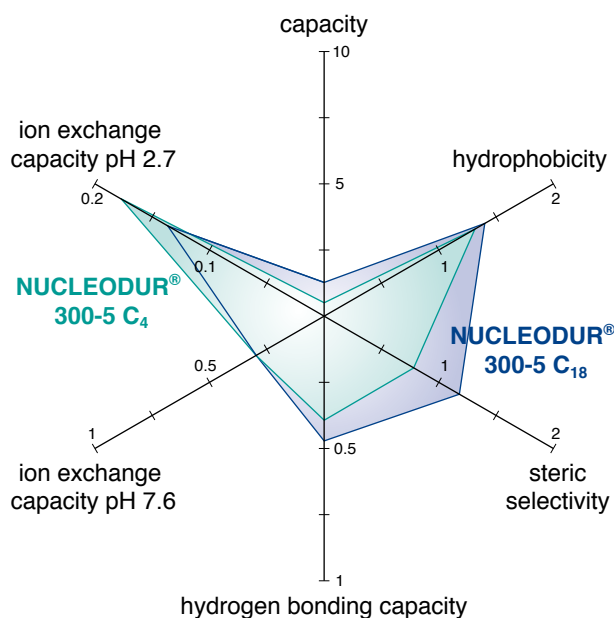
Biological macromolecules like proteins or peptides

USP L1 (C₁₈) / USP L26 (C₄)

wide pore silica for biochromatography



Tanaka plots of NUCLEODUR® wide pore phases



Stability test of NUCLEODUR® 300-5 C₄ ec

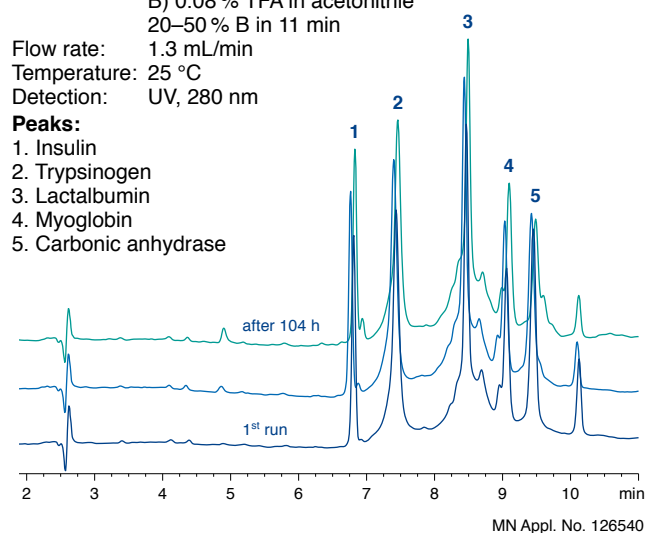
Column: 250 x 4.6 mm NUCLEODUR® 300-5 C₄ ec

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



MN Appl. No. 126540

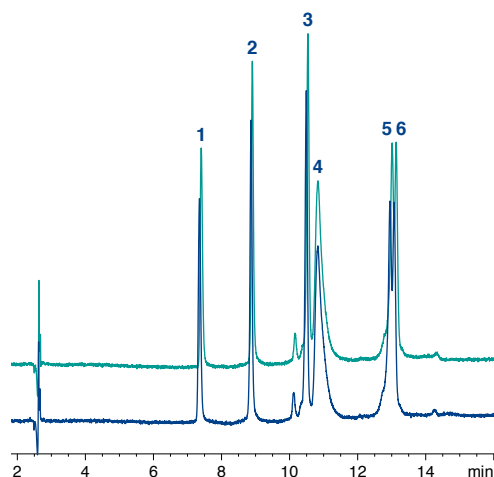
Batch-to-batch reproducibility of NUCLEODUR® C₄ ec and NUCLEODUR® C₁₈ ec wide pore phases

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

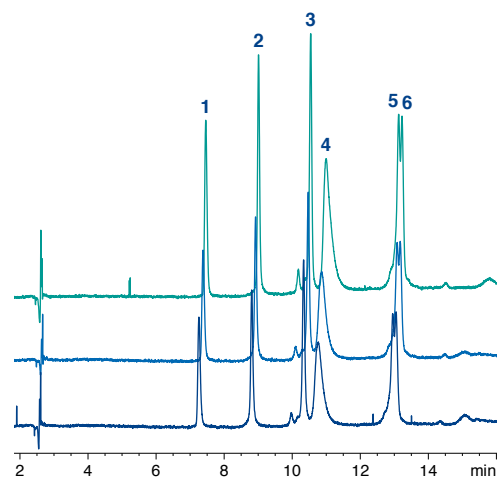
Peaks:

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

NUCLEODUR® 300-5 C₄ ec



NUCLEODUR® 300-5 C₁₈ ec



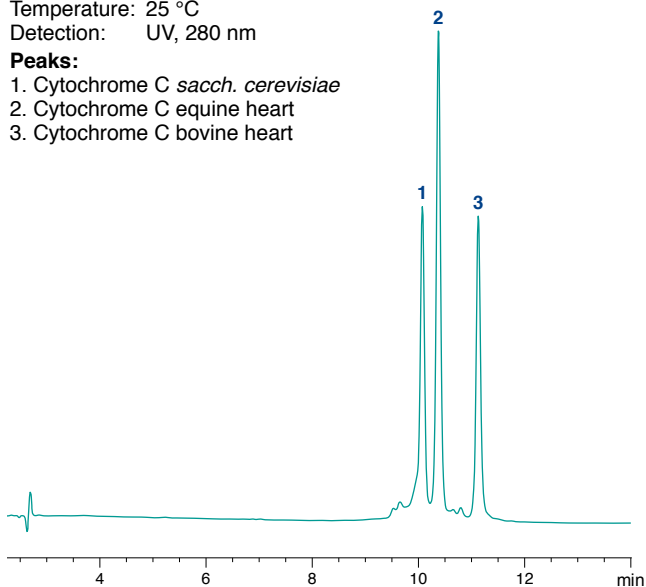
MN Appl. No. 126551/126552

Cytochrome C test

Column: **250 x 4.6 mm NUCLEODUR® 300-5 C₁₈ 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*



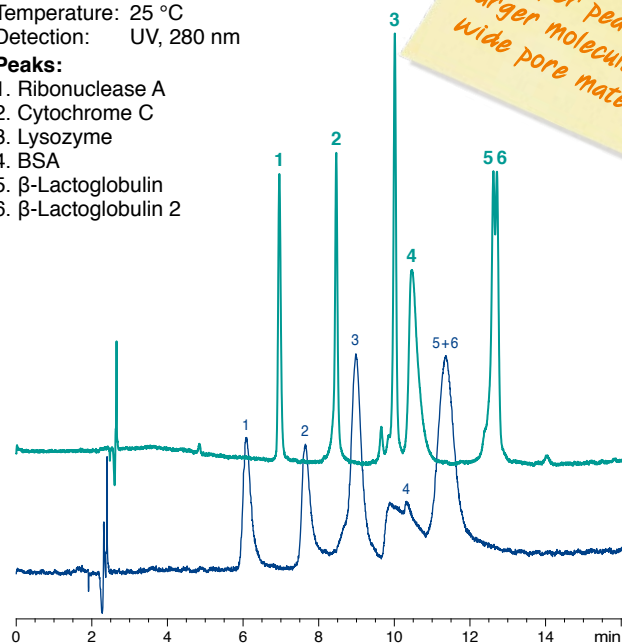
MN Appl. No. 126560

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

Columns: **250 x 4.6 mm NUCLEODUR® 300-5 C₁₈ ec**
250 x 4.6 mm NUCLEODUR® C₁₈ 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



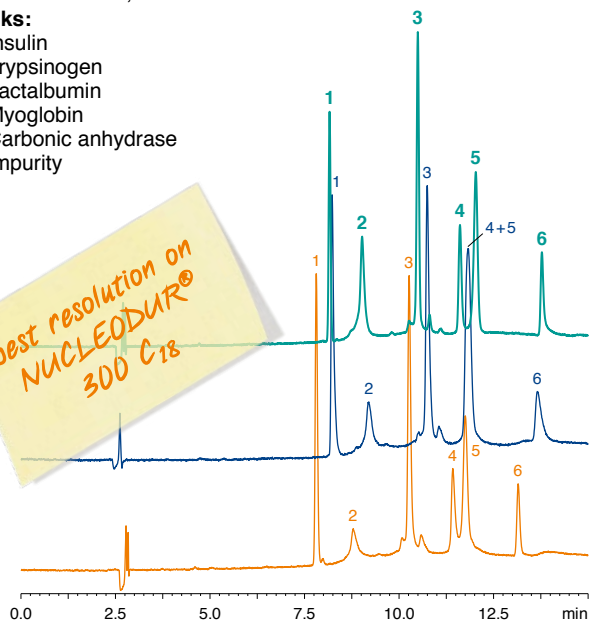
MN Appl. No. 126590

Column comparison for protein separation

Columns: **250 x 4.6 mm NUCLEODUR® 300-5 C₁₈ ec**
250 x 4.6 mm NUCLEOSIL® 300-5 C₁₈
250 x 4.6 mm Jupiter® C₁₈, 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



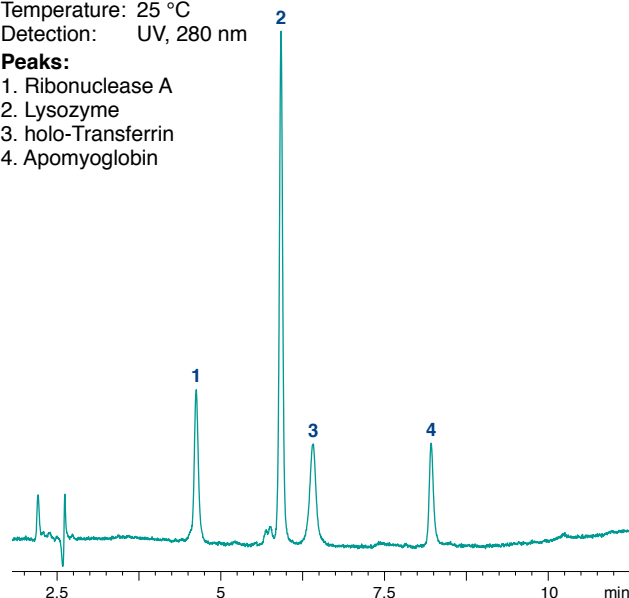
MN Appl. No. 126570

Protein test

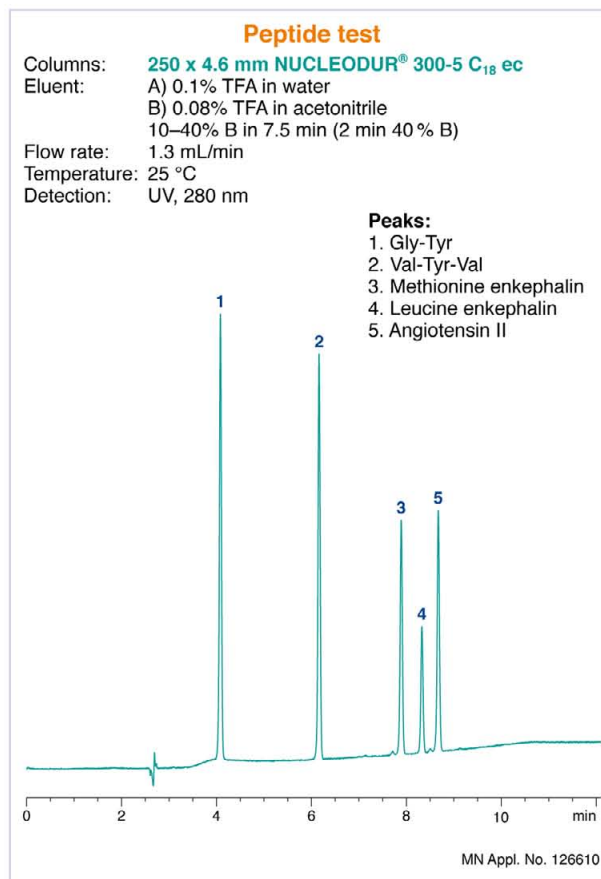
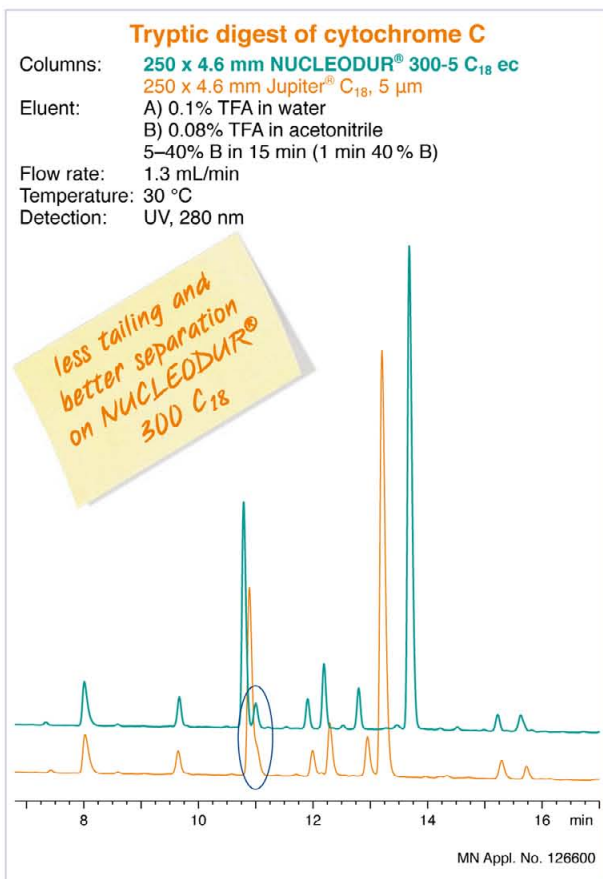
Column: **250 x 4.6 mm NUCLEODUR® 300-5 C₁₈ ec**
 Eluent: A) 0.1 % TFA in water
 B) 0.08 % TFA in acetonitrile
 25–60 % B in 8.5 min (1.5 min 60 % B)
 Flow rate: 1.3 mL/min
 Temperature: 25 °C
 Detection: UV, 280 nm

Peaks:

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



MN Appl. No. 126580



Ordering information · EC columns

NUCLEODUR® 300-5 C₁₈ 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 C₄ 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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MACHEREY-NAGEL



MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany



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1-800-267-8103 • sales@chromspec.com • tech@chromspec.com