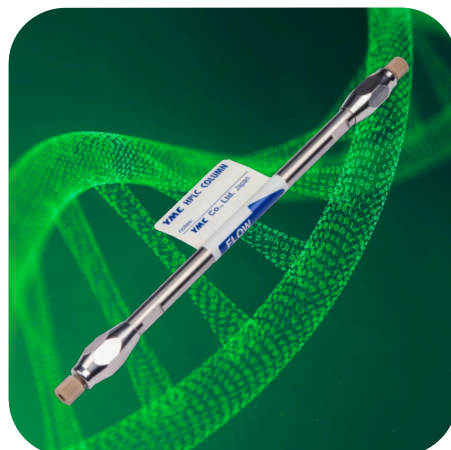




SEC

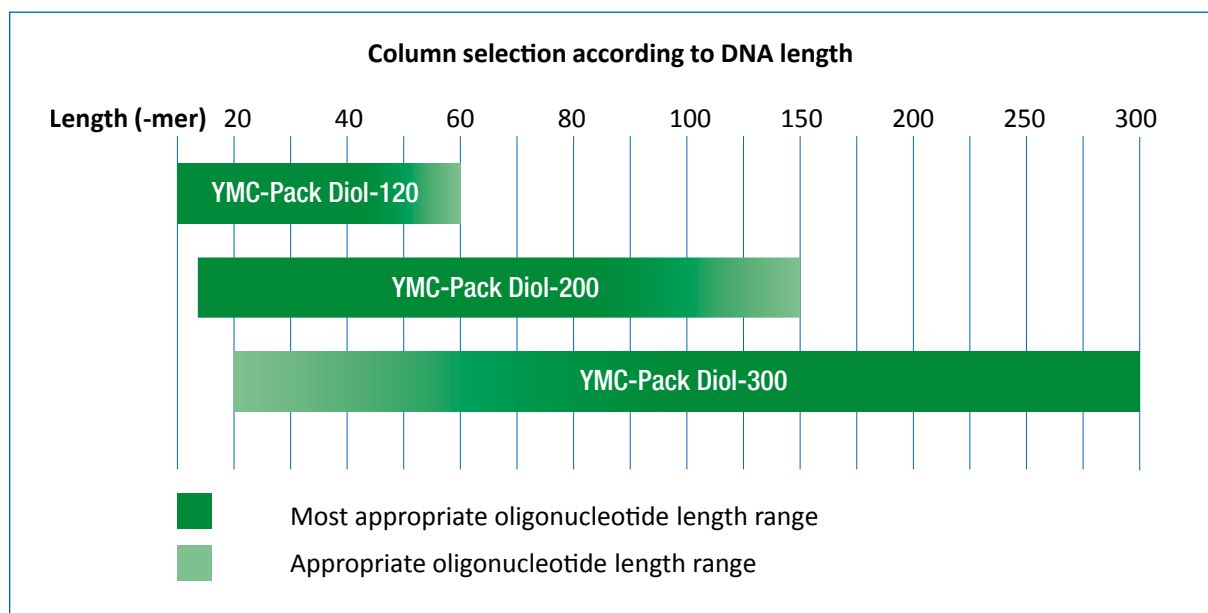


SEC – UHPLC / HPLC Selectivities

Features

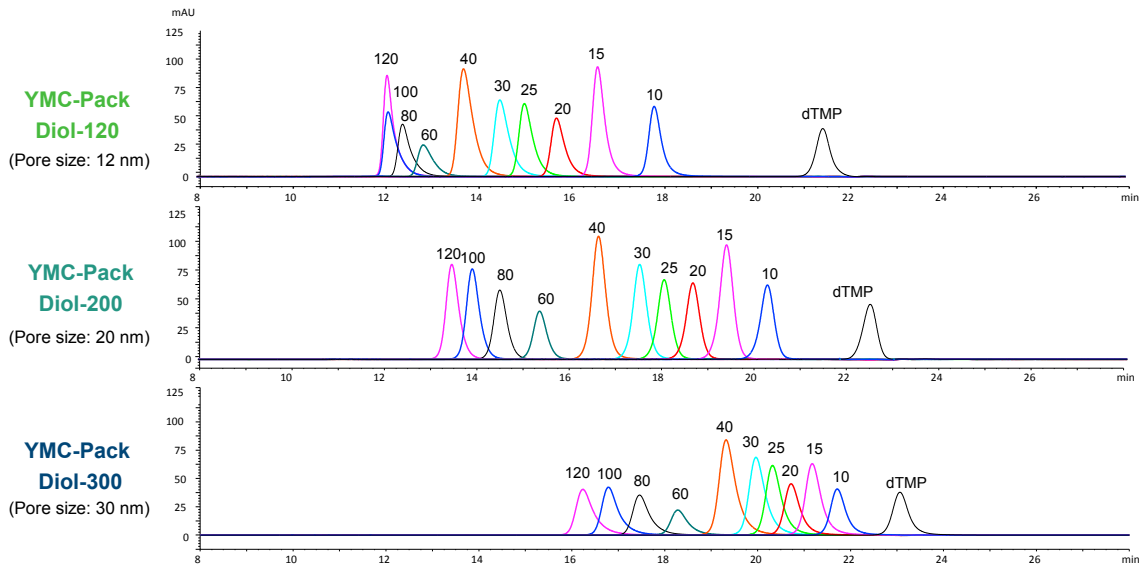
- Excellent reproducibility with minimal secondary interactions
- 2 µm for UHPLC
- Cost-effective

	YMC-Pack Diol-120	YMC-Pack Diol-200	YMC-Pack Diol-300
	For short oligonucleotides	For intermediate oligonucleotides	For longer oligonucleotides
Base particle	Silica		
Particle Size / µm	3, 5	2, 3, 5	2, 3, 5
Pore Size / nm	12	20	30
Modification	Dihydroxypropyl		
Temperature range	40°C		
Pressure limit	2 µm: 45 MPa (6,525 psi); 3/5 µm: 20 MPa (3,000 psi)		

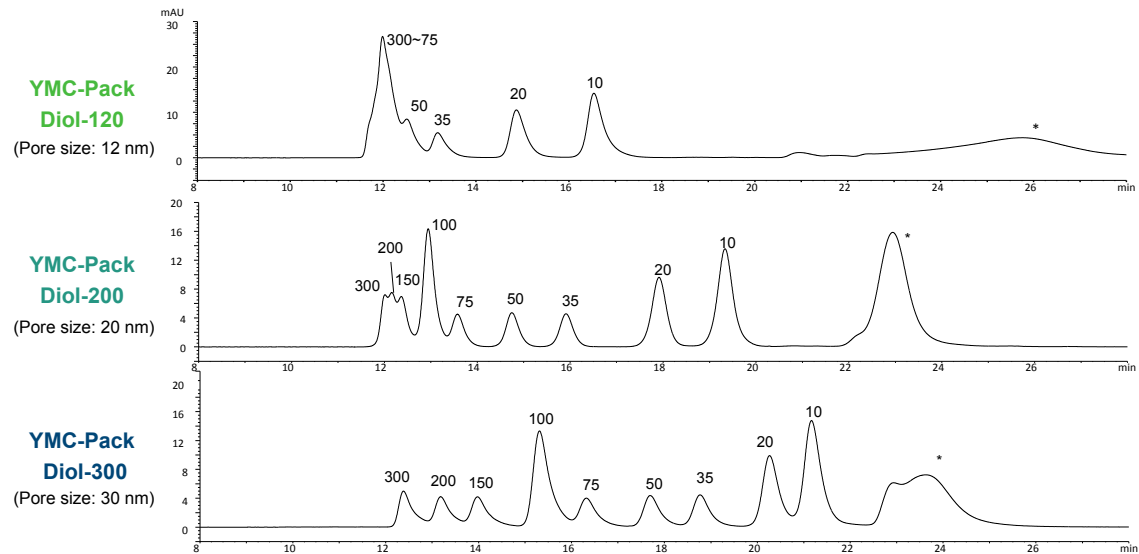


SEC analysis using YMC-Pack Diol columns with different pore sizes

Analysis of ssDNA of 10–120mer length



Analysis of dsDNA of 10–300mer length



Columns: YMC-Pack Diol-120 (5 µm, 12 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-200 (5 µm, 20 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-300 (5 µm, 30 nm) 300 x 4.6 mm ID
 Part Nos.: DL12S05-3046WT
 DL20S05-3046WT
 DL30S05-3046WT
 Eluent: 0.1 M KH₂PO₄-K₂HPO₄ (pH 7.0) containing 0.2 M NaCl

Flow rate: 0.17 mL/min
 Detection: UV at 260 nm
 Temperature: 25 °C
 Injection: 1.0 µL (each 5 nmol/mL)
 Samples: ssDNA (10–120mer +dTMP)
 dsDNA (10–300mer)

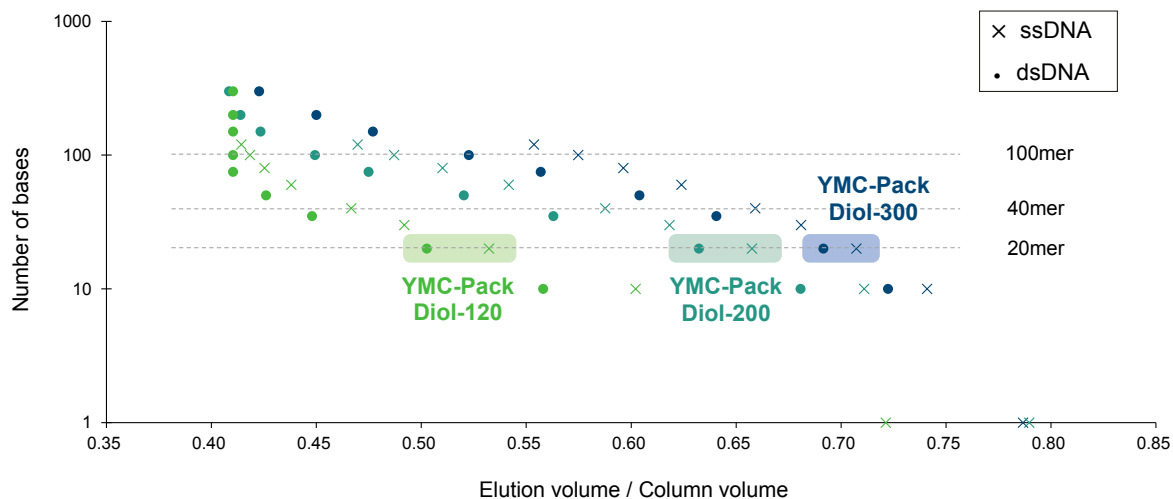
YMC-Pack Diol-120 is best suited for shorter oligonucleotides (10–40mer), whereas YMC-Pack Diol-200 shows the best resolution for oligonucleotides of medium size (30–80mer). Longer oligonucleotides of 60–120mer in length are separated most effectively by YMC-Pack Diol-300.

Similar results are obtained when analysing dsDNA. Small oligonucleotides are separated with higher resolution when smaller pore sizes of 12 and 20 nm are used. Above a length of 50mer, oligonucleotides are unable to penetrate the small pores and elute at the same time. YMC-Pack Diol-200 can resolve oligonucleotides up to a size of 100mer. dsDNA of 150–300mer are only separated by YMC-Pack Diol-300 with the largest pore size of 30 nm. This column also shows the best resolution over a wide range of oligonucleotide lengths.

SEC – Elution volumes

Comparison of the elution volume of ssDNA and dsDNA

Selected corresponding ssDNA and dsDNA pairs of the same number of bases are marked



Columns: YMC-Pack Diol-120 (5 μ m, 12 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-200 (5 μ m, 20 nm) 300 x 4.6 mm ID
 YMC-Pack Diol-300 (5 μ m, 30 nm) 300 x 4.6 mm ID
 Part Nos.: DL12S05-3046WT
 DL20S05-3046WT
 DL30S05-3046WT
 Eluent: 0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
 Flow rate: 0.17 mL/min
 Detection: UV at 260 nm
 Temperature: 25 $^\circ\text{C}$
 Injection: 1.0 μL (each 5 nmol/mL)
 Samples: ssDNA (10–120 mer +dTMP)
 dsDNA (10–300 mer)

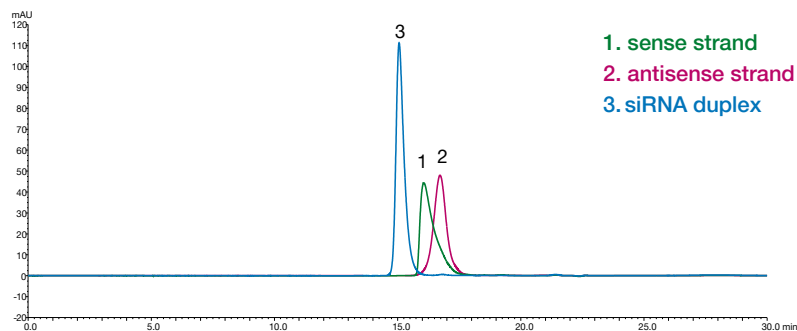
Although dsDNA has the same length as its single-stranded counterpart, the dsDNA elutes at lower elution volumes when separated by SEC. This behaviour is most probably due to the larger hydrodynamic radius of dsDNA compared to ssDNA, which results in faster diffusion through the stationary phase.

Influence of organic modifier on the separation of siRNA duplex and single strands

Effect of acetonitrile on separation of sense strand, antisense strand and siRNA duplex by SEC using YMC-Pack Diol-120

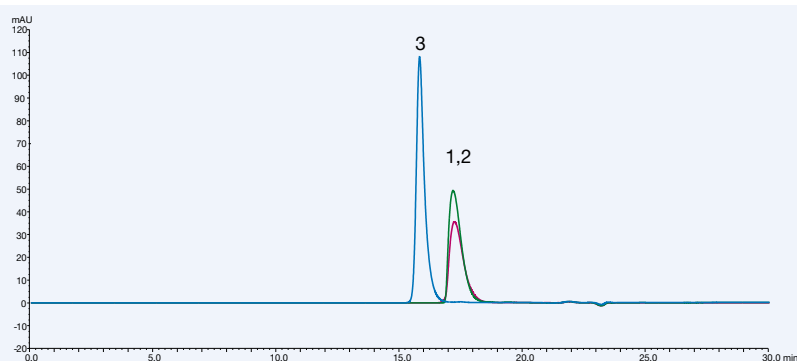
acetonitrile 0%

0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)
containing 0.2 M NaCl



acetonitrile 30%

0.1 M KH_2PO_4 - K_2HPO_4 (pH 7.0)
containing 0.2 M NaCl /
acetonitrile (70/30)



Column: YMC-Pack Diol-120 (5 μm , 12 nm) 300 x 4.6 mm ID
Part No.: DL12S05-3046WT
Flow rate: 0.17 mL/min
Detection: UV at 260 nm
Temperature: 25 $^{\circ}\text{C}$
Injection: 4.0 μL (each 5 nmol/mL)
Sample: Sense strand
Antisense strand
siRNA duplex (Firefly luciferase GL2)

Although the sense and antisense strands have the same molecular weight, their retention times and peak shapes vary slightly. This is probably caused by minor secondary interactions with the stationary phase. If the goal is the separation of siRNA duplex from single strands, these interactions can be overcome with the addition of an organic solvent to the mobile phase.

When 30% acetonitrile is added to the eluent, both single strands elute at the same time and their peak shape is improved.