Preparative Size Exclusion Chromatography for Protein Separation

SRT-10 SEC-300 Prep Column
21.2 x 400 mm (141 mL)
SRT-10 SEC-300 Prep Column

- Column size 21.2 x 400 mm (21.2 x 100mm P/N 225300-21210 + 21.2 x 300 mm P/N 225300-21230)
- Columns are connected with PEEK column coupler (Sepax part # 102002-coupler)
- Instrument GE AKTA explorer
- Pressure: 17 bar at 7 mL/min, 25 bar at 10 mL/min, well below the pressure limit for the AKTA system 100 bar or 250 bar depending on the model.
SEC prep column- BSA loading study

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 3 mL BSA (10, 20, or 30 mg/mL)
**SEC prep column-BSA loading**

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),  
Mobile phase: phosphate buffer, 150mM, pH7.0  
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C;  
Samples: 3 mL BSA (10, 20, or 30 mg/mL)

<table>
<thead>
<tr>
<th>BSA30mg</th>
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<tbody>
<tr>
<td>Peak</td>
<td>RT(min)</td>
<td>Area (mAU*min)</td>
<td>Height (mAU)</td>
<td>W1/2 (min)</td>
<td>Rs</td>
<td>Plates/meter (N/m)</td>
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<tr>
<td>BSA dimer</td>
<td>11.85</td>
<td>101.6082</td>
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<td>Peak</td>
<td>RT(min)</td>
<td>Area (mAU*min)</td>
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<td>W1/2 (min)</td>
<td>Rs</td>
<td>Plates/meter (N/m)</td>
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<td>Height (mAU)</td>
<td>W1/2 (min)</td>
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<td>Plates/meter (N/m)</td>
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<td>BSA</td>
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**SEC prep column – BSA/Uracyl-7 mL/min**

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: **7 mL/min**, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C
Samples: 3 mL BSA(10 mg/mL), Uracyl (0.17 mg/mL)
SEC prep column – BSA/Uracil-10 mL/min

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: **10 mL/min**, 25 bar; Detector: UV 280 nm; Column temperature: 23 °C
Samples: 3 mL BSA (10 mg/mL), Uracil (0.17 mg/mL)
BSA/uracil - overlay 7 and 10 mL/min

Column: SRT-10 SEC-300 (10 µm, 300 Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 °C
Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)

Higher flow rate shortens the separation time while maintaining the resolution.
## SEC prep column-different flow rate

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 °C
Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)

<table>
<thead>
<tr>
<th>flow rate 7mL/min</th>
<th>Peak</th>
<th>RT(min)</th>
<th>Area (mAU*min)</th>
<th>Height (mAU)</th>
<th>W1/2 (min)</th>
<th>Rs</th>
<th>Plates/meter(N / m)</th>
<th>Asymmetry</th>
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<td>Uracil</td>
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<td>4.83</td>
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<th>W1/2 (min)</th>
<th>Rs</th>
<th>Plates/meter(N / m)</th>
<th>Asymmetry</th>
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<tbody>
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<td>315.8112</td>
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<td>Uracil</td>
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<td>4.5</td>
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<td>16609</td>
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SEC prep – QC standard mixture

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C
Samples: 1 or 2 mL Thyroglobulin, BSA, Ribonuclease A (10mg/mL each)

1. Thyroglobulin aggregate
2. Thyroglobulin 670 kD
3. BSA dimer 132 kD
4. BSA monomer 66 kD
5. Ribonuclease A 14 kD
## QC standard separation parameters

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C
Samples: 1 or 2 mL Thyroglobulinm, BSA, Ribonuclease A (10mg/mL each)

<table>
<thead>
<tr>
<th>Injection volume 1mL</th>
<th>Peak</th>
<th>RT(min)</th>
<th>Area ( mAU*min)</th>
<th>Height (mAU)</th>
<th>W1/2 (min)</th>
<th>Rs</th>
<th>Plates/meter(N / m)</th>
<th>Asymmetry</th>
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<tr>
<td>Thyroglobulin aggr</td>
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<td>Ribonuclease A</td>
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<th>Injection volume 2mL</th>
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<th>Area ( mAU*min)</th>
<th>Height (mAU)</th>
<th>W1/2 (min)</th>
<th>Rs</th>
<th>Plates/meter(N / m)</th>
<th>Asymmetry</th>
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<td>Thyroglobulin aggr</td>
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