Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Data Sheet

Eshmuno® CPS resin

High capacity and salt tolerance for recombinant protein purification

Eshmuno® CPS cation exchange chromatography (CEX) resin combines high dynamic binding capacity and separation efficiency in downstream purification processes of recombinant protein feed streams at elevated salt concentrations.

The demonstrated salt tolerance of Eshmuno® CPS resin enables direct loading of high conductivity feed streams (conductivity ≥10 mS/cm), reducing the need of dilution. Direct savings can be made on buffer, time and manufacturing footprint. Streamlined process steps, associated with efficient purification result in an overall improved productivity.

As a strong cation exchanger without hydrophobic groups, Eshmuno® CPS resin allows easy process development with straightforward binding and elution conditions and selection of process parameters.

Benefits

- Outstanding binding capacities at elevated conductivity levels (≥ 10 mS/cm)
- Strong cation exchanger chemistry without hydrophobic groups for easy process development
- Rigid base bead that enables higher flow rates and easier column packing
- Improved productivity through cost and time savings

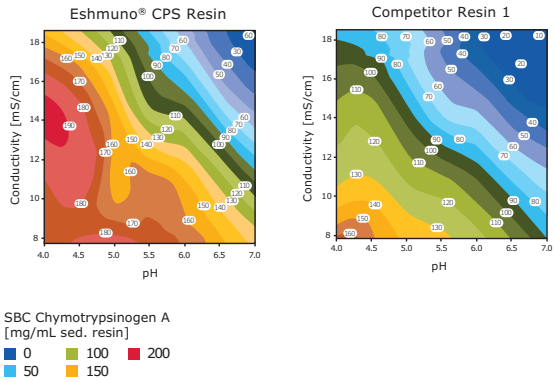
Binding capacity studies with Chymotrypsinogen A

Chymotrypsinogen A with its molecular weight of 25 kDa and pI of 9.1 serves as a suitable model for a relatively small non-mAb recombinant protein with basic pI. Therefore, it is used to demonstrate salt-tolerant binding characteristics of Eshmuno® CPS resin.

Static chymotrypsinogen A binding capacity

Experimental conditions

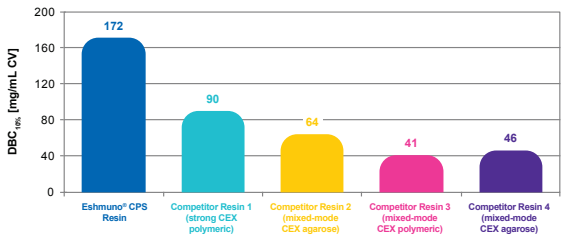
- **Protein concentration:** 10 mg/mL in binding buffer
- **Incubation time:** 15 min
- **Binding buffer:** 25 mM sodium acetate + 25 M sodium phosphate + 50 - 150 mM sodium chloride, adjusted to respective pH and conductivity



Dynamic chymotrypsinogen A binding capacity

Experimental conditions

- **Feed:** 2.5 mg/mL chymotrypsinogen A in buffer A
- **Column size:** 8 mm i.d. x 20 mm
- **Column volume:** 1 mL
- **Residence time:** 4 min
- **Buffer A (equilibration):** 50 mM sodium acetate + sodium chloride, pH 5, adjusted to 12 mS/cm
- Determination of dynamic binding capacity at 10% breakthrough level ($DBC_{10\%}$)



Eshmuno® CPS resin provides significantly higher dynamic binding capacities for the model protein Chymotrypsinogen A at elevated conductivity levels compared to benchmark resins.

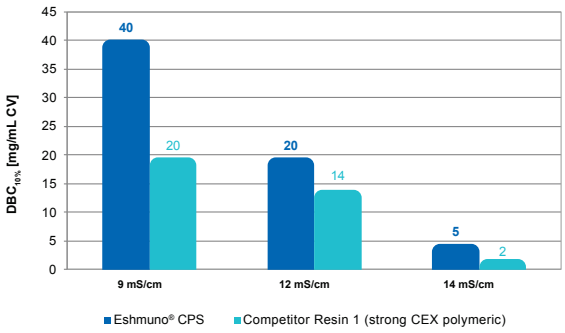
Application 1: Purification of a Fab fragment from E.coli

Purification (capture) runs of a recombinant Fab fragment from E.coli lysate were carried out in order to demonstrate separation efficiencies of Eshmuno® CPS resin for feeds with higher impurity content.

Dynamic binding capacity measurements

Experimental conditions

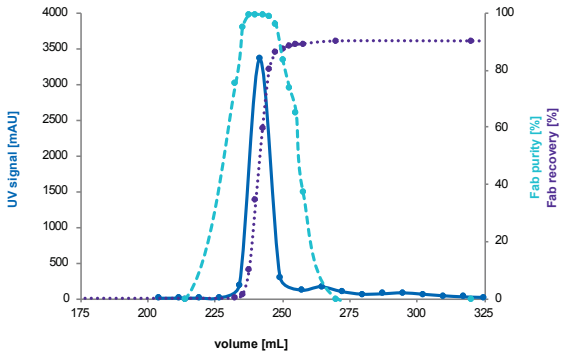
- **Feed:** cleared cell lysate from E. coli cells was adjusted to 9 - 14 mS/cm with 50 mM sodium acetate and spiked with Fab fragment; Fab titer 1 mg/mL, Fab purity 10%
- **Column size:** 8 mm i.d. x 20 mm
- **Column volume:** 1 mL
- **Residence time:** 2 min
- **Buffer A:** 50 mM sodium acetate, pH 4.5, adjusted with sodium chloride to 9 - 14 mS/cm
- Determination of $DBC_{10\%}$



Purification run

Experimental conditions

- **Feed:** cleared cell lysate from E. coli cells was adjusted to 9 mS/cm with 50 mM sodium acetate and spiked with Fab fragment; Fab titer 1 mg/mL; Fab purity 10%
- **Protein load:** 80% of $DBC_{10\%}$ corresponding to 32 mg Fab per mL CV
- **Column size:** 8 mm i.d. x 100 mm
- **Column volume:** 5 mL
- **Residence time:** 2 min
- **Buffer A (equilibration):** 50 mM sodium acetate + 65 mM sodium chloride, pH 4.5, 9 mS/cm
- **Buffer B:** 50 mM sodium acetate + 1 M sodium chloride, pH 4.5
- **Wash:** 10 CV 50 mM sodium acetate, pH 4.5
- **Gradient elution:** 0 - 100% buffer B in 40 CV
- **Analytics:** purity, titer: UV absorption



Purification profile of Fab fragment purity and recovery

Eshmuno® CPS resin showed strong performance in Fab capture from E. coli lysate at much higher capacity than benchmark resins, achieving high purity of and yield of target protein.

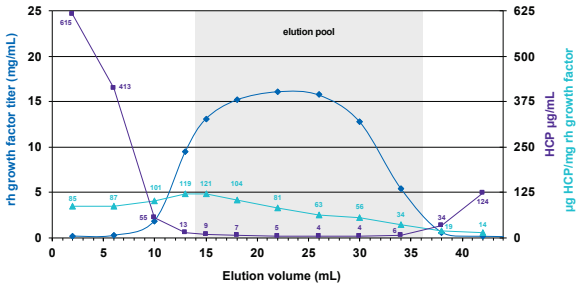
Application 2: Purification of a recombinant human growth factor

Eshmuno® CPS resin was applied for capture of a recombinant human (rh) growth factor (expressed in E. coli as inclusion bodies), with a molecular weight of 23 – 24 kDa and a pI of 10.

Dynamic binding capacity measurements

Experimental conditions

- **Feed:** rh growth factor in refolding buffer, titer 2 mg/mL, pH 8.0, 36 mS/cm
- **Column size:** 8 mm i.d. x 20 mm
- **Column volume:** 1 mL
- **Residence time:** 3 min
- **Buffer A:** A: 50 mM Tris/HCl + 120 mM sodium chloride, pH 8.0
- Determination of $DBC_{10\%}$



Elution profile of rh growth factor and host cell proteins

Purification run

Experimental conditions

- **Feed:** rh growth factor after solubilization and refold present in a mixed buffer system, titer 1.7 mg/mL, pH 8.0, 36 mS/cm
- **Load:** limited to 54% of $DBC_{10\%}$ corresponding to 72 mg rh growth factor per mL CV
- **Column size:** 5 mm i.d. x 200 mm
- **Column volume:** 3.9 mL
- **Residence time:** 3 min
- **Buffer A:** 50 mM Tris/HCl + 120 mM sodium chloride, pH 8.0
- **Buffer B:** 50 mM Tris/HCl + 2 M sodium chloride, pH 8.0
- **Wash:** 10 CV buffer A
- **Gradient elution:** 15% – 100% buffer B in 21 CV, then 2 CV 100% buffer B
- **Analytics:** purity, titer: RP-HPLC
  HCP level: E. coli HCP ELISA

column load	feed	elution pool†					
(mg/mL CV)	HCP/ rh growth factor (ng/mL)	Pool volume (mL)	rh growth factor titer (mg/mL)	Purity by HPLC (%)	Yield by HPLC* (%)	HCP/ rh growth factor (ng/mg)	HCP reduction factor
72	61642	22.0	13.1	95	89	6002	10

* based on rh growth factor recovered from the column
† pool criteria: fractions with purity > 90%

Eshmuno® CPS resin provides high dynamic binding capacity of 120 mg/mL of a recombinant human growth factor even at increased flow rates and an elevated conductivity level of 36 mS/cm. The results of 95% purity and 89% yield of the target protein resin prove strong separation performances of the resin.

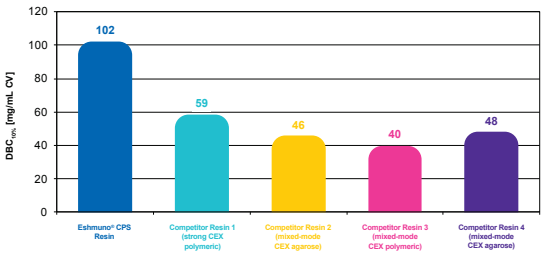
Application 3: Purification of a F(ab')$_2$ fragment from CHO cell culture supernatant

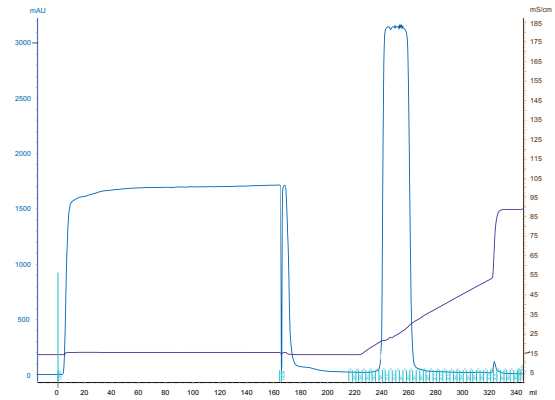
Eshmuno® CPS resin was applied for capture of a F(ab')$_2$ fragment (expressed in CHO cells), with a pI of approx. 7.5-8.6.

Dynamic binding capacity measurements

Experimental conditions

- **Feed:** F(ab´)$_2$ fragment (pI ≈ 7.5 - 8.6) in CHO cell culture supernatant; F(ab´)$_2$ titer 2.5 mg/mL, pH 4.5, 13 mS/cm
- **Column size:** 8 mm i.d. x 20 mm
- **Column volume:** 1 mL
- **Residence time:** 4 min
- **Buffer A:** 50 mM sodium acetate + 80 mM sodium chloride, pH 4.5, 12 mS/cm
- Determination of DBC$_{10\%}$



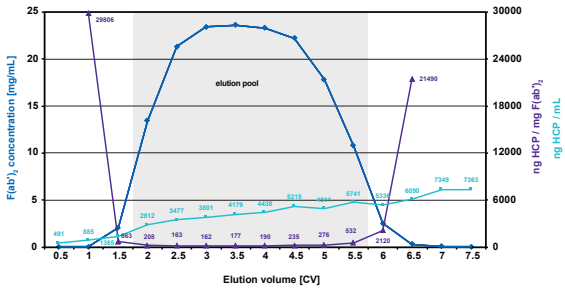


Elution of laod and elution of (Fab')$_2$ fragment with linear salt gradient

Purification run

Experimental conditions

- **Feed:** F(ab´)$_2$ fragment in CHO cell culture supernatant; F(ab´)$_2$ titer 2.5 mg/mL, pH 4.5, 13 mS/cm
- **Load:** limited to 80% of DBC$_{10\%}$ corresponding to 82 mg F(ab')$_2$ per mL CV
- **Column size:** 8 mm i.d. x 100 mm
- **Column volume:** 5 mL
- **Residence time:** 4 min
- **Buffer A:** 50 mM sodium acetate + 80 mM sodium chloride, pH 4.5, 12-13 mS/cm
- **Buffer B:** 50 mM sodium acetate + 1 M sodium chloride, pH 4.5
- **Wash:** 5 CV buffer A
- **Gradient elution:** 0 - 50% buffer B in 20 CV
- **Analytics:** purity, titer: SE-HPLC
  HCP level: CHO HCP ELISA



Elution profile of F(ab')$_2$ fragment and host cell proteins

column load	feed	elution pool*					
80% DBC$_{10\%}$ (mg/mL CV)	HCP/F(ab')$_2$ (ng/mg)	Pool volume (mL)	F(ab')$_2$ titer (mg/mL)	Purity by SE-HPLC (%)	Yield by SE-HPLC (%)	HCP/F(ab')$_2$ (ng/mg)	HCP reduction factor
82	27546	20.0	19.5	> 99	95	220	125

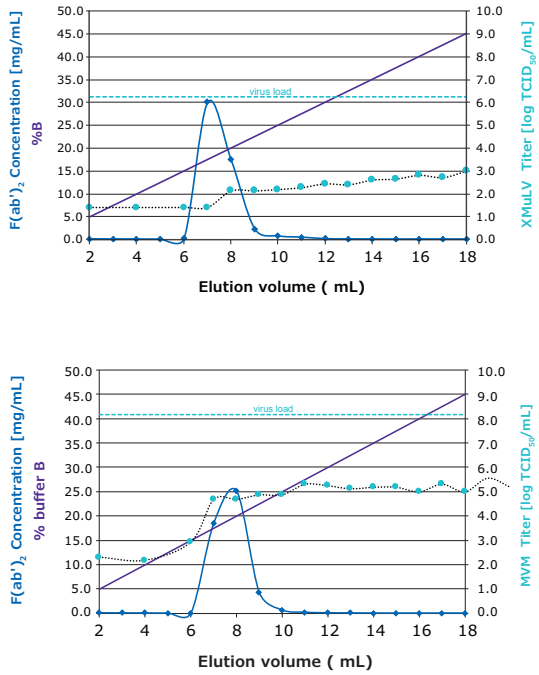
Eshmuno® CPS resin was shown to efficiently capture F(ab')$_2$ from CHO cell culture supernatant. High process loading (82 mg/mL) was used and efficient HCP clearance was achieved via salt gradient elution.

Virus Removal Study

In order to check how Eshmuno® CPS resin can contribute to virus removal in a downstream purification process, virus spiking studies of two different viruses (retrovirus XMuLV and parvovirus MVM) were performed using F(ab')$_2$ in CHO cell culture supernatant as feed stream.

Experimental conditions

- **Feed:** F(ab´)$_2$ fragment in CHO cell culture supernatant spiked with virus, F(ab´)$_2$ titer 2.5 mg/mL, pH 5.0, 10 mS/cm
- **Load:** 50 mg F(ab')$_2$ per mL CV
- **Column size:** 8 mm i.d. x 100 mm
- **Column volume:** 5 mL
- **Residence time:** 2 min
- **Buffer A:** 50 mM sodium acetate + 75 mM NaCl, pH 5.0, 10 mS/cm
- **Buffer B:** 50 mM sodium acetate + 1 M NaCl, pH 5.0
- **Wash:** 5 CV buffer A
- **Gradient elution:** 0 - 50% buffer B in 20 CV
- **Analytics:** purity, F(ab')$_2$ titer: SE-HPLC
  Virus titer: $TCID_{50}$ infectivity assay



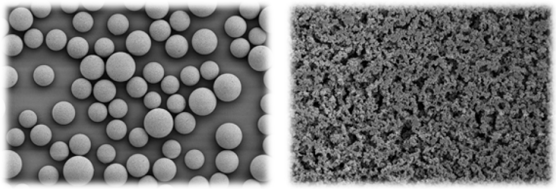
Elution profile of F(ab')^2 fragment and XMuLV and MVM

Type of Virus	Virus Load [$TCID_{50}$]	Virus in Elution Pool [$TCID_{50}$]	LRV in F(ab')$_2$ elution pool
xMuLV (retrovirus)	Run 1: 3.3 x 10^8	Run 1: 1.5 x 10^3	**5.4 ± 0.0**
	Run 2: 3.3 x 10^8	Run 2: 1.5 x 10^3	
MVM (parvovirus)	Run 1: 1.4 x 10^8	Run 1: 5.5 x 10^5	**2.3 ± 0.1**
	Run 2: 1.5 x 10^8	Run 2: 9.7 x 10^5	

It was demonstrated that Eshmuno® CPS resin has great potential in contributing significantly to parvovirus and retrovirus removal in a downstream purification process scheme.

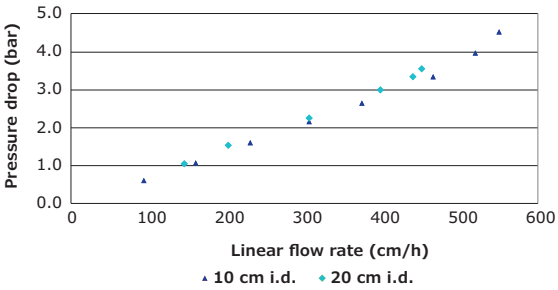
Proven technology

Eshmuno® CPS resin is a member of our high performance Eshmuno® platform, which is a family of chromatography resins designed to meet the demands of highly productive downstream processes. Eshmuno® base beads are composed of a hydrophilic polyvinyl ether polymer that enables high flow rates and therefore shorter processing times. Eshmuno® ion exchange resins also make use of our well-established tentacle ligand technology that provides a flexible multipoint interaction between the protein and resin resulting in higher binding capacities and superior selectivity.



SEM pictures of Eshmuno® CPS resin

Eshmuno® CPS resin can be easily packed into production-scale columns, either by simple flow packing or axial compression. The pressure-flow curves for 10cm and 20cm i.d. columns at 20cm bed height are shown below demonstrating linear scalability.



Flow packed in 0.15 M NaCl, 20 cm bed height, 14% compression, running buffer: 0.15 M NaCl

Process Development

Eshmuno® CPS resin is available in pre-packed, ready-to-use, disposable columns. MiniChrom columns can be used for lab-scale process development with any standard chromatography system, whereas RoboColumns® can be utilized for high-throughput process development in conjunction with a chromatography robot. These small scale columns are the ideal tool for performing initial resin screening, scaling and optimization studies.

	Eshmuno® CPS Chromatography Resin
Type of chromatography	Strong cation exchanger
Functional group	Sulfoisobutyl
Base material	Surface grafted rigid hydrophilic polyvinylether polymer
Mean particle size (d50)	50 µm
pK value	<1
pH stability	pH 2 to 14
Mechanical stability	8 bar
Linear flow rate	up to 300 cm/h (< 3.0 bar net pressure) 20 x 10 cm i.d. column, 12% - 14% compression equivalent to 1.14 to 1.16 compression factor, 150 mM NaCl as mobile phase
Storage conditions	20% EtOH + 150 mM NaCl solution, ambient temperature
Shipping solution	20% EtOH + 150 mM NaCl solution

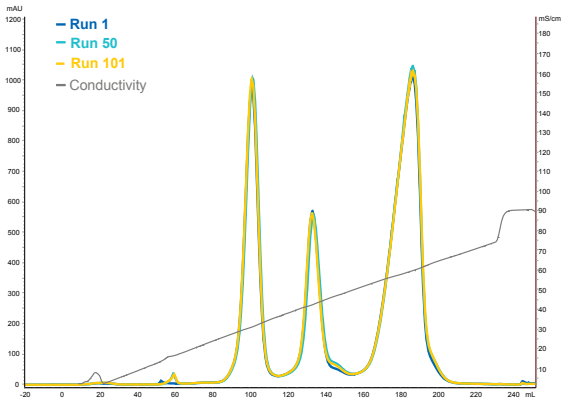
CIP Stability

Eshmuno® CPS resin can easily be cleaned or sanitized and is compatible over a wide range of pH conditions. It has excellent stability under both alkaline and acidic conditions. No significant differences in the separation of a three protein mixture were observed after 100 CIP cycles (60 minutes exposure to 1.0 M sodium hydroxide per cycle).

100 cycle study

Experimental conditions

- **Feed:** solution of Chymotrypsinogen A (14.3 mg/mL), Cytochrome C (7.2 mg/mL) and lysozyme (21.5 mg/mL) in buffer A (total protein concentration 43.0 mg/mL)
- **Load:** 5 mL sample volume corresponding to 215 mg total protein
- **Column:** 16 mm i.d. x 100 mm
- **Column volume:** 20.1 mL
- **Residence time:** 4 min
- **Buffer A:** 20 mM sodium phosphate, pH 6.0
- **Buffer B:** 20 mM sodium phosphate + 1 M NaCl, pH 6.0
- **Wash:** 1 CV Buffer A
- **Gradient elution:** 0 - 80% Buffer B in 10.4 CV
- **CIP:** 2 CV 1M NaOH at 20 cm/h (residence time 30 min)
- **Re-equilibration:** 1 CV buffer B + 5 CV buffer A



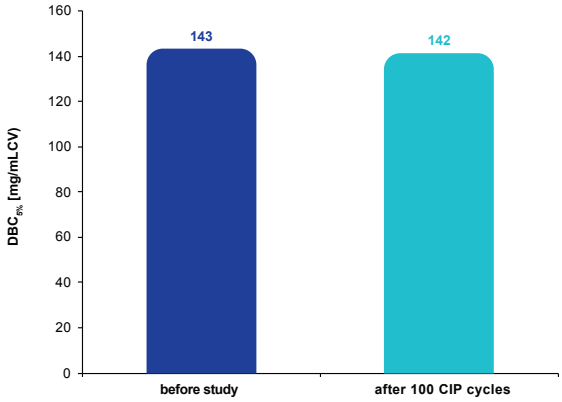
Separation of chymotrypsinogen A, cytochrome C and lysozyme on Eshmuno® CPS resin, overlaid chromatograms of run 1 (blue), run 50 (cyan), and run 101 (yellow)

Dynamic binding capacity measurements

Experimental conditions

- **Feed:** 5 mg/mL lysozyme in buffer A
- **Column:** 16 mm i.d. x 100 mm
- **Column volume:** 20.1 mL
- **Residence time:** 1 min
- **Buffer A:** 20 mM sodium phosphate, pH 6.0
- Determination of $DBC_{5\%}$

The resolution of protein separation remains unchanged over a multitude of runs. The dynamic protein binding capacity has not been altered after 100 chromatographic cycles.



Ordering information

Description	Catalog Number
Eshmuno® CPS, 10 ml	**1.20084.0010**
Eshmuno® CPS, 100 ml	**1.20084.0100**
Eshmuno® CPS, 500 ml	**1.20084.0500**
Eshmuno® CPS, 5L	**1.20084.5000**
MiniChrom prepacked column with Eshmuno® CPS resin, 1ml 8x20mm	**1.25164.0001**
MiniChrom prepacked column with Eshmuno® CPS resin, 5ml 8x100mm	**1.25165.0001**
RoboColumn® prepacked column with Eshmuno® CPS, 0.2ml 8PC 5x10mm	**1.25166.0001**
RoboColumn® prepacked column with Eshmuno® CPS, 0.6ml 8PC 5x30mm	**1.25167.0001**
Buffer Preparation	**Catalog Number**
di-Potassium hydrogen phosphate anhydrous EMPROVE® EXPERT Ph Eur,BP,USP	**137010**
Sodium chloride EMPROVE® EXPERT Ph Eur,BP,JP,USP	**137017**
Sodium dihydrogen phosphate dihydrate EMPROVE® EXPERT Ph Eur,BP,USP,JPE	**137018**
Sodium hydroxide pellets EMPROVE® EXPERT Ph Eur,BP,JP,NF	**137020**
Sodium hydroxide solution 1 mol/L EMPROVE® EXPERT	**137031**
Tris(hydroxymethyl)aminomethane (Trometamol) EMPROVE® ESSENTIAL Ph Eur,BP,JPC,USP	**108386**
Tris(hydroxymethyl)aminomethane (Trometamol) high purity EMPROVE® EXPERT Ph Eur,BP,JPC,USP,ACS	**108307**
Tris(hydroxymethyl)aminomethane hydrochloride EMPROVE® EXPERT	**108219**
Hydrochloric acid 1 mol/L EMPROVE® EXPERT	**110165**
Acetic acid 1 mol/L EMPROVE® EXPERT	**137035**
Acetic acid 30% EMPROVE® EXPERT Ph Helv	**137047**
Acetic acid (glacial) 100% EMPROVE® EXPERT Ph Eur,BP,JP,USP	**137000**
Sodium acetate anhydrous EMPROVE® EXPERT USP	**137046**
Sodium acetate trihydrate EMPROVE® EXPERT Ph Eur,BP,JP,USP	**137012**
Column Cleaning & Storage	**Catalog Number**
Ethanol 20% EMPROVE® EXPERT	**480910**
Ethanol 20 % (v/v) with 150 mMol/L sodium chloride solution EMPROVE® EXPERT	**480940**
Sodium hydroxide solution 0,1 mol/L EMPROVE® EXPERT	**137058**
Sodium hydroxide solution 0,5 mol/L EMPROVE® EXPERT	**137060**
Ethanol absolute suitable for use as excipient EMPROVE® exp Ph Eur,BP,JP,USP	**100986**

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