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Preparation, Separation,
Filtration & Monitoring Products

Data Sheet

Eshmuno® CMX Chromatography Resin

A highly selective mixed mode chromatography resin for difficult to purify mAbs, ADCs and fusion proteins

Eshmuno® CMX chromatography resin is a mixed mode chromatography resin built on the proven Eshmuno® resin technology. This innovative resin combines weak cation exchange properties with hydrophobic interaction, providing high selectivity for Monoclonal Antibody (mAb), fusion protein and Antibody Drug Conjugate (ADCs) purification as well as separation of low molecular weight impurities and Host Cell Proteins (HCPs).

Eshmuno® CMX chromatography resin enables users to

- **Intensify processes and decrease costs** by reducing the number of chromatographic steps and buffer consumption
- **Improve performance** with higher recovery rates, high selectivity and superior dynamic binding capacity
- **Improve the user experience** with a broad operational window, simplified process development and easy column packing due to a rigid base bead

Proven Eshmuno® Technology

Eshmuno® CMX chromatography resin is a member of the Eshmuno® family of high-performance chromatography resins designed to meet the demands of highly productive downstream purification processes. Eshmuno® ion exchange resins carry an innovative surface tentacle structure which is able to bind target substances much more effectively. The resins combine this superior tentacle technology with the advantages of a rigid hydrophilic polyvinyl ether base matrix, enabling high flow rates and shorter processing times.

Application: Monoclonal Antibody and Fusion Protein Purification

Improvements in development processes for mAbs and fusion proteins have led to higher productivity and higher titers, increasing the number of high and low molecular weight impurities and the complexity of the purification process.

Traditional downstream processes for mAb and fusion proteins include capture, 2nd purification and final polishing steps. Studies using Eshmuno® CMX chromatography resin have shown that this process can be reduced from three to two chromatographic steps due to the high selectivity of the resin (Figure 1); this in turn reduces process time and costs while maintaining purity (Figure 2).

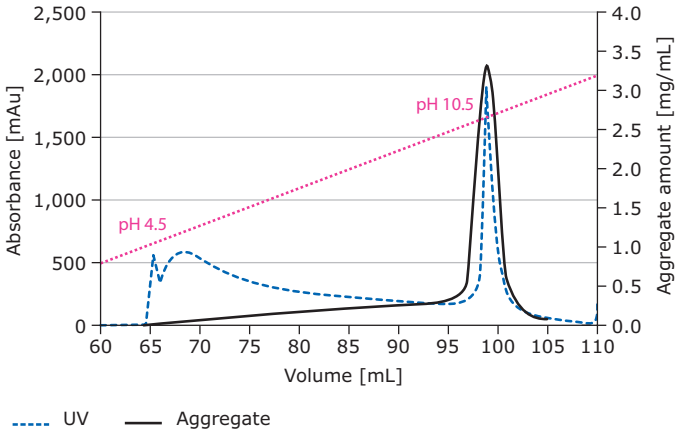


Figure 2 – Elution profile of an Fc fusion protein containing up to 30% aggregates on Eshmuno® CMX chromatography resin using linear pH gradient.

Compared to a traditional chromatography resin (Table 1), Eshmuno® CMX chromatography resin triples process yield while maintaining product purity.

Traditional downstream process

Capture		2nd Purification step		Polishing		Process	
Purity [%]	Yield [%]	Purity [%]	Yield [%]	Purity [%]	Yield [%]	**Purity [%]**	**Yield [%]**
81.06	96.39	92.54	36.18	97.37	36.56	**97.37**	**12.75**

Downstream process using Eshmuno® CMX chromatography resin

Capture		Polishing		Process	
Purity [%]	Yield [%]	Purity [%]	Yield [%]	**Purity [%]**	**Yield [%]**
82.94	100	97.15	41.26	**97.15**	**41.26**

Table 1 – Comparison of yield, fusion protein purification, traditional chromatography resin versus Eshmuno® CMX chromatography resin.

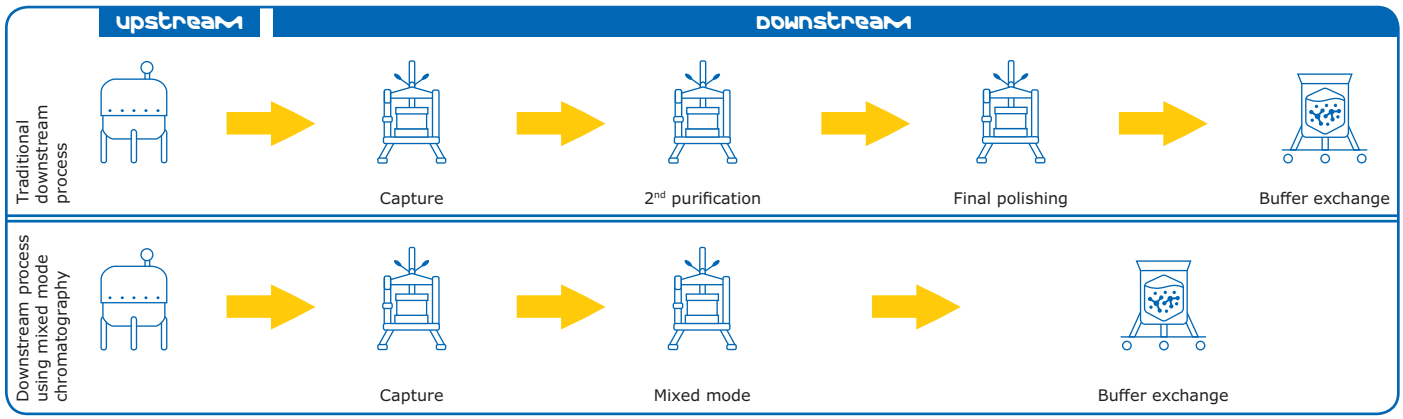


Figure 1 – Comparison of traditional downstream process and mixed mode chromatography process for fusion proteins.

Application: ADC Polishing

The quality attributes of ADCs include those associated with the antibody, the small molecule conjugate form and the small molecule drug moiety. The Drug-to-Antibody Ratio (DAR) is a critical quality attribute, as a high DAR may affect the safety profile of the ADC, while a low DAR may decrease its efficacy.

Eshmuno® CMX chromatography resin can be used to remove undesired low DAR and high DAR species, enabling the best pharmacokinetics, efficacy, stability and tolerability.

The mixed mode cation exchange step follows the conjugation step to separate antibody drug conjugate species, and usually operates in a bind and elute mode at a pH between 4 to 5 and 150 to 250 mM NaCl (Figure 3). Under these conditions, most of antibody drug conjugates will bind to the resin and separation of low or too high DAR/antibody drug conjugate species will be achieved in selecting the optimal elution conditions. Elution conditions include the pH change and/or conductivity change, that increases the selectivity of the mixed mode cation exchange resin. This is achieved operating a gradient mode or in a step elution mode, where buffers having different pH and/or conductivity are applied (Figure 4).

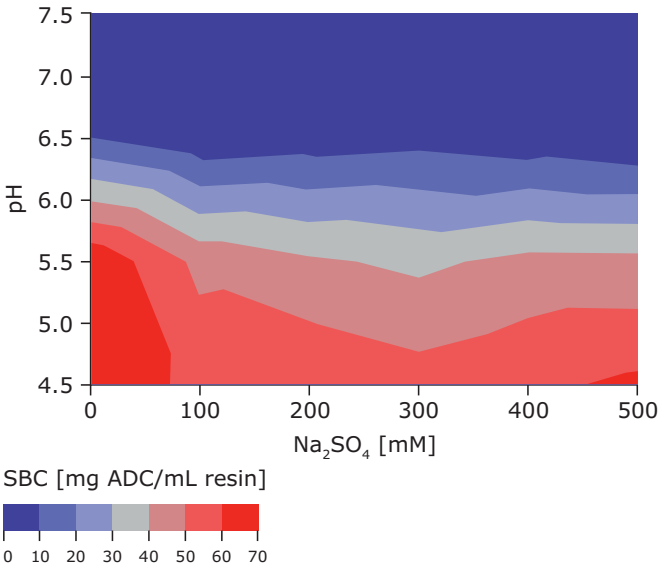


Figure 3 – Quantitative example of antibody drug conjugate molecule binding under a range of pH and conductivity conditions. 50 mM acetate and 50 mM phosphate buffer system was used to achieve the pH with different Na_2SO_4 amounts. Static incubation was done for 120 minutes in 0.4 mL sample suspension.

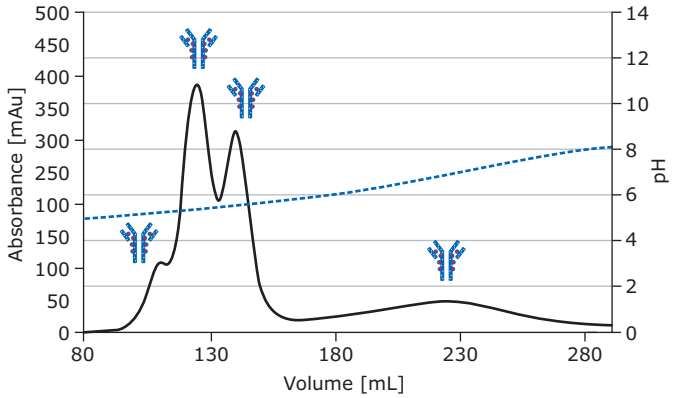


Figure 4 – ADC specie separation using Eshmuno® CMX resin column loaded @30 mg protein/mL packed bed and using pH gradient elution. Solid line represents the UV signal trace and the dashed line represents the pH signal trace (e.g. secondary axis). Antibody symbols represent the DAR species from DAR2 to DAR8.

Application: Removal of Low Molecular Weight Impurities and HCPs

The improvements in mAb and fusion protein manufacturing processes have led to higher productivity and titers, accompanied by increased levels of low molecular weight impurities and HCPs that pose a challenge for the downstream process. As shown in Figure 5, Eshmuno® CMX chromatography resin is effective at reducing the level of these impurities.

Figure 5a

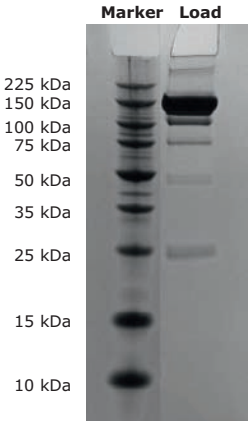
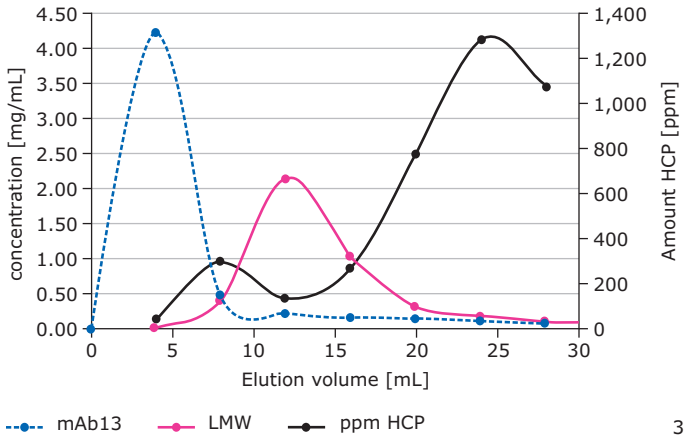


Figure 5 – Monoclonal antibody containing sample (165 kDa) with high quantities of low molecular weight impurities (100 kDa) and HCPs (5a). Separation of the monoclonal antibody-containing sample from low molecular weight impurities and HCPs using pH gradient (5b).

Figure 5b



Enhanced Ease of Use

With a broad operational window, Eshmuno® CMX chromatography resin enables use of various pH levels and conductivity to obtain high product recovery (Figure 6). It is also the only mixed mode resin enabling elution of hydrophobic mAbs (Figure 7).

Figure 6a

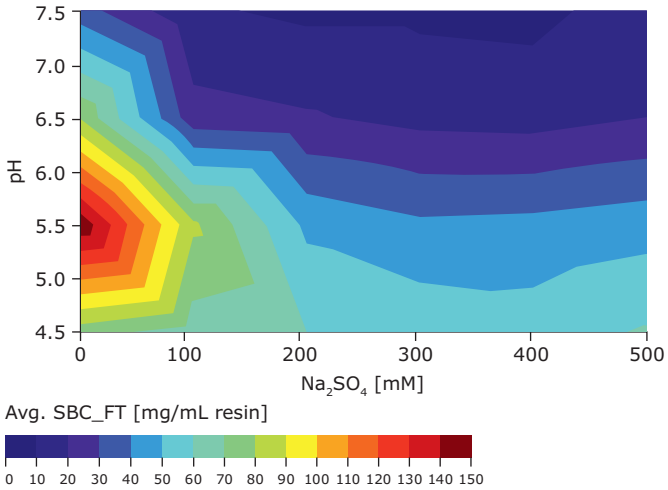


Figure 6b

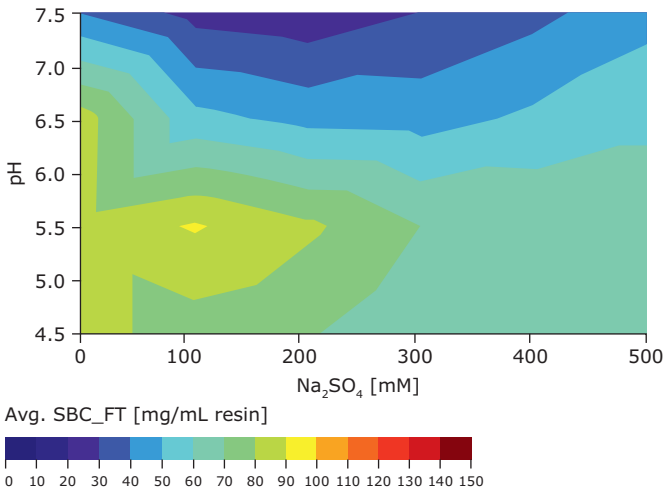


Figure 6 – Two-dimension antibody static binding capacity values in varying pH and Na_2SO_4 concentration window after 2 hours of incubation. The values are average values in mg protein/mL settled resin calculated from the flow through fractions:
6a) Eshmuno® CMX resin,
6b) competitor mixed mode resin.

Figure 7a

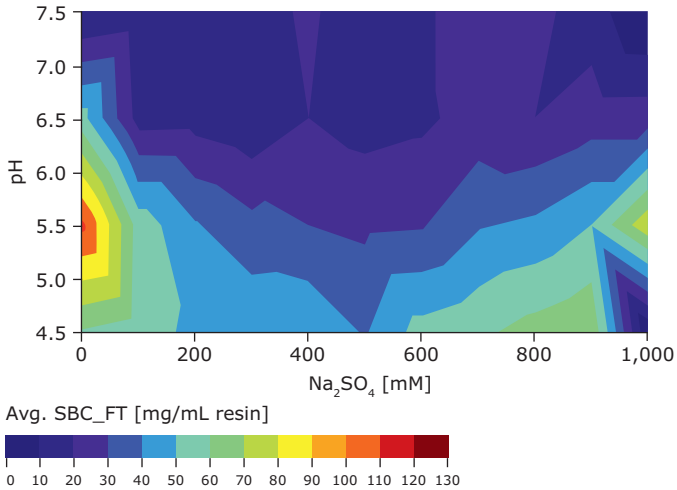


Figure 7b

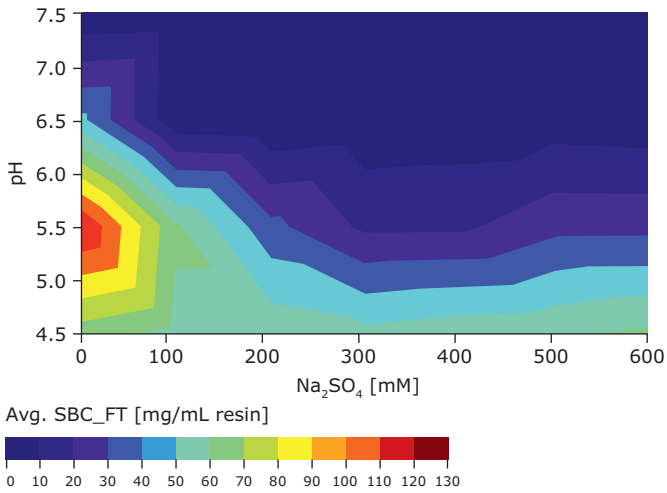


Figure 7c

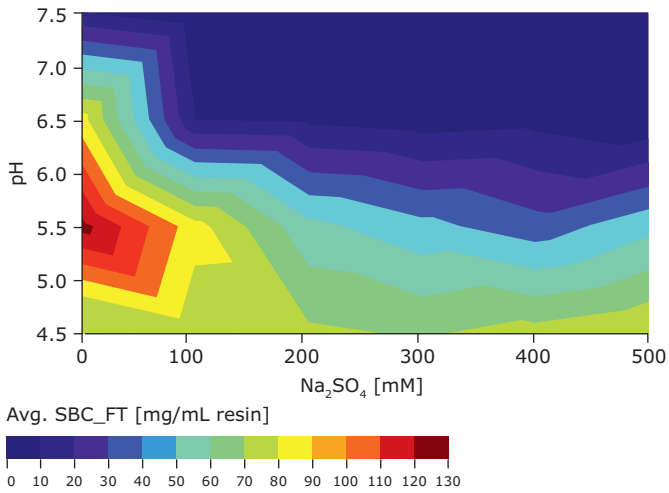
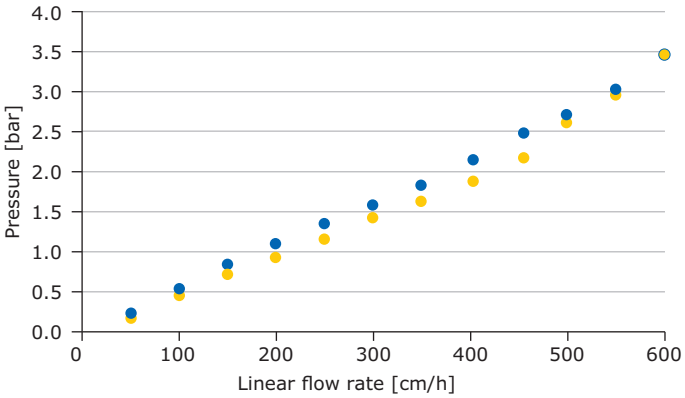


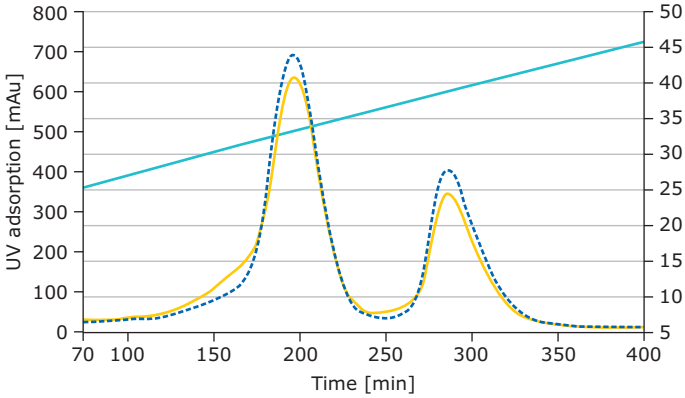
Figure 7 – Two-dimension antibody static binding capacity values in varying pH and Na_2SO_4 concentration window after two hours of incubation. The values are average values in mg protein/mL. Eshmuno® CMX settled resin calculated from the flow through fractions hydrophobic antibody 7a, antibody 7b and hydrophobic antibody 7c.

As shown in Figures 8 and 9, Eshmuno® CMX chromatography resin can be easily packed due to the rigid nature of the bead and easily sanitized providing robust clean in place (CIP) stability.



- Packing 1: EthOH 20% – 12% compession
- Packing 2: NaOH 0.1M – 14.5% compession

Figure 8 – Pressure versus flow curve for Eshmuno® CMX resin packed in 20 cm ID column using EthOH versus NaOH.



- conductivity (mS/cm) trace – cycle 1
- conductivity (mS/cm) trace – cycle 106
- UV signal trace – cycle 1
- UV signal trace – cycle 106

Figure 9 – Portion of chromatographic separation obtained after the first and the 106th cycle applying chymotrypsinogen A and cytochrome C. Each cycle is an individual chromatographic run containing column equilibration, sample loading, column washing, conductivity gradient elution, 1M NaOH cleaning and column regeneration steps.

Technical Information

Type of chromatography	Mixed Mode
Functional group	COO-(weak cation exchange) and alkyl-functional groups
Base material	Surface grafted rigid hydrophilic polyvinylether polymer
Mean particle size (d_{50})	50 µm
Dynamic protein binding capacity (4 min residence time, 5% BT)	60 mg pIgG/mL packed resin
Ionic capacity	120 µMol/mL, settled resin
pK value	<1
pH stability during operations	In working conditions (proteins/contaminants binding and elution): pH 2 to 12 In cleaning and sanitization: pH 0 to 14
Mechanical stability	8 bar
Linear flow rate	up to 300 cm/h 20 × 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, temperature between +2 °C to +30 °C
Shipping solution	20% EtOH/150 mM NaCl solution

Ordering information

Eshmuno® CMX resin, 10 mL	1.20650.0010
Eshmuno® CMX resin, 100 mL	1.20650.0100
Eshmuno® CMX resin, 500 mL	1.20650.0500
Eshmuno® CMX resin, 5 L	1.20650.5000
MiniChrom prepacked column with Eshmuno® CMX resin, 1 mL 8 × 20 mm	1.25185.0001
MiniChrom prepacked column with Eshmuno® CMX resin, 5 mL 8 × 100 mm	1.25186.0001
RoboColumn® prepacked column with Eshmuno® CMX resin, 0.2 mL 8PC 5 × 10 mm	1.25187.0001
RoboColumn® prepacked column with Eshmuno® CMX resin, 0.6 mL 8PC 5 × 30 mm	1.25188.0001

Buffer Preparation

Citric acid anhydrous powder EMPROVE® ESSENTIAL Ph Eur,BP,JP,USP,E 330,FCC	100241
Sodium dihydrogen phosphate monohydrate EMPROVE® EXPERT BP,USP	137093
Glycine cryst. EMPROVE® EXPERT Ph Eur,BP,JP,USP	100590
N-[Tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid buffer substance TAPS	108320
DI-SODIUM SUCCINATE ANHYDROUS FOR SYNTHESIS	818601
Sodium hydroxide pellets EMPROVE® EXPERT Ph Eur,BP,JP,NF	137020
Sodium hydroxide solution 32% EMPROVE® EXPERT	137023
Sodium chloride EMPROVE® EXPERT Ph Eur,BP,JP,USP	137017
Potassium dihydrogen phosphate cryst., EMPROVE® ESSENTIAL Ph Eur,BP,JPC,NF,E 340	104871
di-Potassium hydrogen phosphate anhydrous, EMPROVE® ESSENTIAL Ph Eur,BP,E 340	105101

Column Cleaning & Storage

Ethanol 20% EMPROVE® EXPERT	480910
Ethanol 20% (v/v) with 150 mMol/L sodium chloride solution EMPROVE® EXPERT	480940
Guanidinium chloride EMPROVE® EXPERT	137037
Sodium hydroxide solution 0.1 mol/L EMPROVE® EXPERT	137058
Sodium hydroxide solution 0.5 mol/L EMPROVE® EXPERT	137060
Ethanol absolute EMPROVE® EXPERT Ph Eur,BP,JP,USP	100986
2-Propanol 70% (v/v) EMPROVE® EXPERT USP	137040
2-Propanol EMPROVE® ESSENTIAL Ph Eur,BP,JP,USP	100995
Benzyl alcohol EMPROVE® EXPERT Ph Eur,BP,JP,NF,ACS	137043

Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Notes

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt
Germany

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To place an order or receive technical assistance, please visit **MerckMillipore.com/contactPS**

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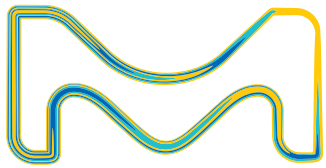

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