HiScreen™ Capto™ adhere ImpRes HiTrap™ Capto adhere ImpRes, 1 ml

Capto adhere ImpRes is a multimodal BioProcess™ chromatography medium for polishing of monoclonal antibodies and other recombinant/native proteins by packed bed chromatography.

The combination of the high flow agarose used for all Capto products and the small particle size of Capto ImpRes results in excellent pressure-flow properties as well as enhanced resolution. Capto adhere ImpRes provides:

- Innovative selectivity of the multimodal ligand
- High resolution chromatography media based on the well-established Capto ImpRes platform
- High productivity enabling improved process economy

HiScreen Capto adhere ImpRes (4.7 ml) and HiTrap Capto adhere ImpRes (1 ml) are prepacked columns for small scale purifications, as well as optimization of methods and parameters, such as sample load and binding conditions. HiScreen Capto adhere ImpRes and HiTrap Capto adhere ImpRes columns provide fast, reproducible and easy separations in convenient formats. The columns are best utilized when they are connected to liquid chromatography systems such as ÄKTA™.

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Please read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, please refer to the Safety Data Sheets.

1 Product description

HiScreen column characteristics

HiScreen columns are made of biocompatible polypropylene that does not interact with biomolecules. The columns are delivered with stoppers at the inlet and outlet. The arrow on the column label indicates the column orientation and the recommended flow direction, see Figure 1.

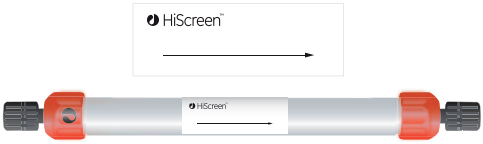

Fig 1. HiScreen column

For easy scaling-up and when a higher bed height is required, two columns can easily be connected in series using a union to give 20 cm bed height (see Section *Scaling up*).

Note: *HiScreen columns cannot be opened or refilled*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 1. Characteristics of HiScreen column

Column volume (CV)	4.7 ml
Column dimensions	0.77 × 10 cm
Column hardware pressure limit	8 bar (0.8 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 2 lists the characteristics of HiTrap columns.

Fig 2. HiTrap, 1 ml column.

Note: *HiTrap columns cannot be opened or refilled.*

Note: *Make sure that the connector is tight to prevent leakage.*

Table 2. Characteristics of HiTrap columns.

Column volume (CV)	1 ml
Column dimensions	0.7 × 2.5 cm
Column hardware pressure limit	5 bar (0.5 MPa)

Note: *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

Supplied Connector kit with HiTrap column

Connectors supplied	Usage	No. supplied
Union 1/16" male/luer female	For connection of syringe to HiTrap column	1
Stop plug female, 1/16"	For sealing bottom of HiTrap column	2, 5 or 7

Properties of Capto adhere ImpRes

Multimodal functionality

Capto adhere ImpRes has a ligand with multimodal functionality (see figure below). The multimodal functionality gives a different selectivity compared to traditional ion exchangers and also provides the possibility of operating in different regions with respect to pH and conductivity.

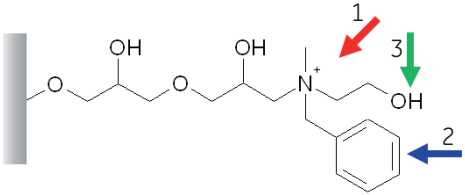

Fig 3. The Capto adhere ImpRes ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interactions (1), hydrophobic interactions (2) and hydrogen bonding (3). The chromatography medium is designed for polishing, and is based on the high flow agarose base matrix with a small bead size, which gives good pressure-flow properties and high resolution.

Polishing

Capto adhere ImpRes is designed for post protein A polishing of monoclonal antibodies. Removal of leached protein A, aggregates, host cell proteins, nucleic acids and viruses from monoclonal antibodies is performed in either flow-through or bind-elute mode. In flow-through mode the antibody passes directly through the column while the contaminants are bound, while in bind-elute mode the high resolution of the small bead is utilized for separation of antibody monomer from contaminants during elution.

High flow velocities

In spite of the small bead size of Capto adhere ImpRes (average size is 40 µm), the rigid high flow agarose matrix allows high flow velocities (see figure below).

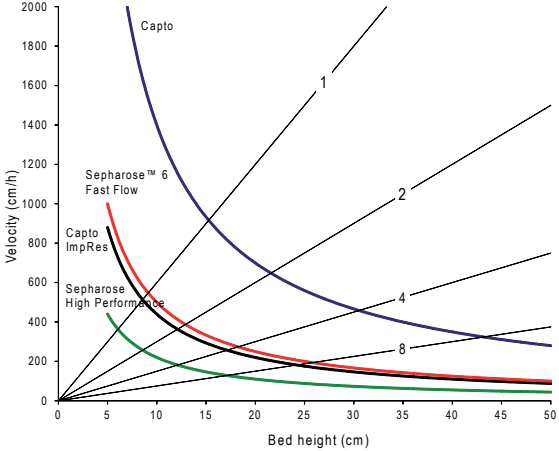

Fig 4. The highly rigid Capto ImpRes matrix allows a large window of operation (area below the curves) at large-scale. Data correspond to a 1 m diameter column at 20°C and viscosity equivalent to water. Gray contours (1, 2, 4, 8) give the residence time in the column in minutes.

Characteristics

The highly cross-linked agarose base matrix gives the medium high chemical and physical stability. Characteristics such as capacity, elution behavior and pressure/flow properties are unaffected by the solutions commonly used in process chromatography and cleaning procedures.

Table 3. Characteristics of Capto adhere ImpRes

Matrix	highly cross-linked agarose
Functional group	multimodal strong anion exchanger
Particle size (d_{50v}) [1]	36 to 44 µm
Total ionic capacity	0.08 to 0.11 mmol Cl^-/ml medium
Recommended flow velocity [2]	100 to 300 cm/h (HiScreen) and 25 to 50 cm/h (HiTrap)
Maximum flow velocity [2]	300 cm/h
pH stability [3]	
Working range	3 to 12
Cleaning-in-place	2 to 14
Working temperature [4]	4°C to 30°C
Chemical stability [5]	All commonly used aqueous buffers, 1 M acetic acid, 1 M sodium hydroxide
Storage	4°C to 30°C in 20% ethanol

[1] d_{50v} is the average particle size of the cumulative volume distribution.

[2] Water at room temperature. See also Table 6.
For viscous buffers and samples the flow velocity must be optimized and starting with a low flow rate is recommended in order not to exceed pressure limits.

[3] Working range: pH interval where the medium can be operated without significant change in function.

Cleaning-in-place: pH stability where the medium can be subjected to cleaning-in-place without significant change in function.

[4] Capto adhere ImpRes can be used under cold-room conditions, but the capacity for some key contaminants may decrease.

[5] No significant change in nitrogen and carbon content after 1 week storage in 1 M NaOH at 40°C.

2 Optimization

Aim

The aim of designing and optimizing a method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. For optimization of binding conditions, pH and conductivity (salt concentration) should be screened.

Screening

When using Capto adhere ImpRes, protein binding can be expected at pH values lower than those used with traditional ion exchangers due to the contribution from non-electrostatic interactions. The multimodal nature of the ligand results in different interaction modes, electrostatic *versus* hydrophobic, depending on buffer conditions and target protein characteristics. A thorough screening of conditions is therefore strongly recommended on Capto adhere ImpRes in order to optimize conditions for binding and elution.

Workflow

The recommended workflow is described in the figure below. It starts with screening of conditions in high throughput formats, followed by optimization, preferably by applying Design of Experiments (DoE), in small columns and finally scale-up to large columns.

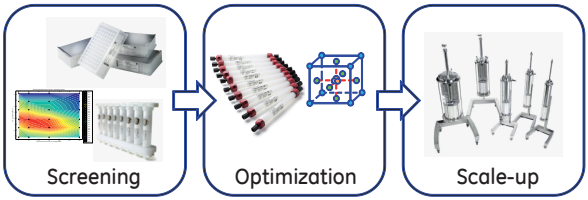

Fig 5. Recommended workflow during process development.

Prepacked chromatography media

Prefilled PreDictor™ plates or PreDictor RoboColumn™ are recommended for condition screening while prepacked HiScreen™ columns are recommended for optimization. Optimization in columns is preferentially done by using Design of Experiments (DoE). DoE can also be used to evaluate plate data, but often raw data analysis of plate data is enough to find conditions that can be used during optimization in packed bed. For experimental setup, data handling, analysis and evaluation of raw data from plates, the Assist software from GE Healthcare will be useful.

An example of screening and optimization of a bind-elute application on Capto adhere ImpRes is found in Application note *MAb polishing in Bind & Elute mode using Capto adhere ImpRes* (29-0273-38).

3 Operation

Prepare buffers

The buffer species and buffer concentration are important for reproducible and robust methods. The table below shows suitable buffers for ion exchange chromatography and suggested starting concentrations.

Users of ÄKTA systems with BufferPrep or BufferPro functionality can select from a range of buffer recipes to conveniently screen media over a range of pH values and conductivities.

Table 4. Recommended buffers for ion exchange chromatography

pH interval	Buffer [1, 2]	Concentration [3]
4 to 5.5	Acetate	20 to 100 mM
3 to 6.5	Citrate	20 to 200 mM
5.5 to 6.5	Bis-Tris	20 to 50 mM
6 to 7.5	Phosphate	50 to 200 mM
7.5 to 9	Tris	20 to 50 mM
8.8 to 10.6	Glycin-NaOH	20 to 100 mM

1 The choice of buffer systems and salts may influence both yield and contaminant clearance.

2 Buffers in the interval 5.5 to 8 will normally be most efficient for contaminant removal.

3 Conductivity can be adjusted by addition of salt or by varying the buffer concentration.

Prepare the sample

Step	Action
1	Adjust the sample to the composition of the start buffer, using one of these methods: • Dilute the sample with start buffer. • Exchange buffer using a HiPrep™ 26/10 Desalting, HiTrap Desalting or PD-10 Desalting column (see table below).
2	Filter the sample through a 0.45 µm filter or centrifuge immediately before loading it to the column. This prevents clogging and increases the life time of the column when loading large sample volumes.

Note: *For larger volumes of feed, sample preparation is preferably performed by diafiltration or directly by adjustment of pH and conductivity.*

Table 5. Prepacked columns for desalting

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml[1] 1.75 to 2.5 ml[2]	3.5 ml[1] up to 2.5 ml[2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml[1] 0.2 to 0.5 ml[2]	1.0 ml[1] up to 0.5 ml[2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml[1] 0.75 to 1.0 ml[2]	1.5 ml[1] up to 1.0 ml[2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

Recommended flow rates

The pressure-flow specification for the Capto adhere ImpRes medium in large columns with negligible wall support is >220 cm/h in a 20 cm bed height at 3 bar and a buffer viscosity comparable to the one of water. The residence time used should however match those appropriate for the final intended scale of the process and this should be considered when running HiScreen and HiTrap columns. Recommended flow rates for different operations are found in the tables below.

Table 6. Recommended flow rates using HiScreen Capto adhere ImpRes

Type of operation	Flow rate (ml/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration [1]	≤ 2.3	≤ 300	≥ 2
Wash [1]	≤ 2.3	≤ 300	≥ 2
Sample load	0.78 to 1.55	100 to 200	3 to 6
Cleaning-in-place [2]	≤ 0.78	≤ 100	≥ 6

Table 7. Recommended flow rates using HiTrap Capto adhere ImpRes

Type of operation	Flow rate (ml/min)	Flow velocity (cm/h)	Residence time (min)
Equilibration [1]	≤ 1.9	≤ 300	≥ 0.5
Wash [1]	≤ 1.9	≤ 300	≥ 0.5
Sample load	0.16 to 0.32	25 to 50	3 to 6
Cleaning-in-place [2]	≤ 0.16	≤ 25	≥ 6

[1] Stated flow rates are for buffers with the same viscosity as water at 20°C. For solutions with higher viscosities, e.g., 20% ethanol, lower flow rates should be used.

[2] The Cleaning-in-place should be done in at least 3 column volumes with a total contact time of at least 15 min. See also Section Cleaning-in-place (CIP).

Purification

Collect fractions throughout the separation.

Flow rate: See the tables above for recommended flow rates for different operations.

Column tubing: Choose the optimal tubing kit for the column and the application you intend to run. (i.d.: 0.25, 0.50 or 0.75 [mm]). A tubing with wider inner diameter gives broader peaks whereas a tubing with a smaller inner diameter gives higher back pressure.

Column and sample preparation

Step	Action
1	Before connecting the column to a system: • Remove the two stoppers from the HiScreen column. • Remove the stopper in the top and the snap-off in the bottom of the HiTrap column.
2	Connect the column to the system with a drop-to-drop connection to avoid introducing air into the column. ***Note:*** *To prevent leakage, make sure that the connectors are tight. Use fingertight 1/16" connector (28-4010-81).*
3	Wash with 1 column volume (CV) of distilled water. This step removes the ethanol and avoids precipitation of buffer salts upon exposure to ethanol. Since 20% ethanol has higher viscosity than water, the recommended flow rate for this step is: • 1.2 ml/min for HiScreen (150 cm/h) • 1.0 ml/min for HiTrap (150 cm/h) ***Note:*** *This step can be omitted if precipitation is not likely to be a problem.*
4	Equilibrate the column with at least 5 CV start buffer or until the UV baseline, eluent pH and conductivity are stable.
5	Adjust the sample to the chosen starting pH and conductivity, see table 5.

Column run flow-through mode

Step	Action
1	Load sample onto the column. Collect fractions for analysis of yield and purity. ***Note:*** *Target protein is in the flowthrough.*
2	Wash with start buffer until the UV trace of the flowthrough returns to near baseline and collect together with the flow-through fractions.
3	Regenerate column to remove bound material with a low pH strip. ***Note:*** *0.1-0.5 M acetic acid is recommended.*
4	Perform a CIP to elute and clean the column, see Section *Cleaning-in-place (CIP).*
5	Equilibrate with 5-10 CV starting buffer or until the UV baseline, pH, and conductivity reach the required values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with starting buffer until the conductivity and pH values are stable. ***Note:*** *Do not exceed the maximum recommended flow velocity (see Table 3) or the column hardware pressure limit (see Tables 1 and 2).*

Column run bind-elute mode

Step	Action
1	Load sample onto the column. Collect fractions for analysis of yield and purity. ***Note:*** *Target protein is bound.*
2	Wash out unbound material with start buffer.
3	Elute bound material and collect elution fractions for analysis of yield and purity.
4	Regenerate column to remove bound material with a low pH strip. ***Note:*** *0.1-0.5 M acetic acid is recommended.*
5	Perform a CIP to elute and clean the column, see Section *Cleaning-in-place (CIP).*
6	Equilibrate with 5-10 CV starting buffer or until the UV baseline, pH, and conductivity reach the required values. The equilibration step can be shortened by first washing with a high concentration buffer to obtain approximately the desired pH value and then washing with starting buffer until the conductivity and pH values are stable. ***Note:*** *Do not exceed the maximum recommended flow velocity (see Table 3) or the column hardware pressure limit (see Tables 1 and 2).*

4 Cleaning-in-place (CIP)

General description

Correct preparation of samples and buffers should maintain columns in good condition. However, reduced performance, increased back pressure or blockage indicates that the column medium needs cleaning.

CIP removes very tightly bound, precipitated or denatured substances from the medium. If such contaminants are allowed to accumulate, they may affect the chromatographic properties of the prepacked column, reduce the capacity of the medium and, potentially, come off in subsequent runs. If the fouling is severe, it may block the column, increase back pressure and affect the flow properties.

CIP should be performed regularly to prevent the build-up of contaminants and to maintain the capacity, flow properties and general performance of prepacked columns. CIP is usually performed after the elution.

It is recommended to perform a CIP:

- When an increase in back pressure is seen.
- If reduced column performance is observed.
- Before first time use or after long term storage.
- Between runs when the same column is used for purification of different proteins to prevent possible cross-contamination.
- After every run with real feed.

CIP protocol

The nature of the sample will ultimately determine the final CIP protocol so the CIP procedure below may require optimization. NaOH concentration, contact time and frequency are typically the main parameters to vary during the optimization of the CIP.

For increased contact time and due to the viscosity of the CIP solutions it is recommended to use a lower flow rate than during the purification, see tables 6 and 7.

The CIP procedure below removes common contaminants.

Step	Action
1	Wash with at least 2 column volumes (CV) of 2 M NaCl.
2	Wash with at least 3 CV 1 M NaOH with at least 15 min contact time.
3	Wash with at least 2 CV 2 M NaCl.
4	Wash with at least 2 CV distilled water.
5	Wash with 5 CV start buffer or until eluent pH and conductivity have reached the required values

5 Scaling up

After optimizing the method at laboratory scale, the process is ready for scaling up. For quick small scale-up of purification, either two HiTrap columns (one in the top of the other) or two HiScreen columns can conveniently be connected in series with a union (18-1120-93) to give increased bed height. Note that the back pressure will increase. This can easily be addressed by lowering the flow rate.

Factors such as clearance of critical impurities may change when column bed height is modified and should be validated using the final bed height.

Scaling up is otherwise typically performed by keeping bed height and linear flow velocity (cm/h) constant while increasing bed diameter and volumetric flow rate (ml/min or l/h).

Bulk medium is available for further scaling up, see *Ordering information*.

A general description of the scaling up procedure is described below.

Step	Action
1	Select bed volume according to required sample load. Keep sample concentration constant.
2	Select column diameter to obtain the desired bed height. The excellent rigidity of the high flow base matrix allows for flexibility in choice of bed heights.
3	The larger equipment used when scaling up may cause some deviations from the method optimized at small scale. In such cases, check the buffer delivery and monitoring systems for time delays or volume changes.

6 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Figure below. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

Note: *Exceeding the flow limit (see Table 3) may damage the column.*

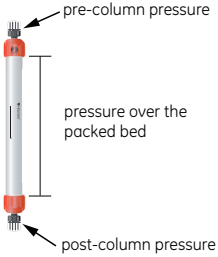

Fig 6. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). The pre-column pressure limit is the column hardware pressure limit (see Tables 1 and 2).

The maximum pressure the packed bed can withstand depends on medium characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

Step	Action
1	Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2	Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3	Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Tables 1 and 2). Replace the pressure limit in the software with the calculated value. The actual pressure over the packed bed (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

Note: *Repeat the procedure each time the parameters are changed.*

7 Storage

Wash with 2 column volumes (CV) of distilled water followed by 2 CV 20% ethanol.

Store at 4°C to 30°C. Do not freeze.

Make sure that the column is tightly sealed to avoid drying out.

8 Troubleshooting

Problem	Possible cause/*corrective action*
High back pressure during the run	The column is clogged. *Clean the column, see Section Cleaning-in-place (CIP).* High viscosity of solutions. *Use lower flow rate.*
Unstable pressure curve during sample loading	Air bubbles trapped in the sample pump. *If possible, degas the sample using a vacuum degasser.*
Gradual decrease in yield	Insufficient elution and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Gradual increase in CIP peaks	Sub-optimal elution conditions and CIP. *Optimize the elution conditions, the CIP protocol and/or perform CIP more frequently.*
Reduced column performance despite optimized elution and CIP	*Change to a new column. The longevity of the column depends mainly on the sample and sample preparation.*

9 Ordering information

Product	Quantity	Code No.
HiScreen Capto adhere ImpRes	1 × 4.7 ml	17-3715-20
HiTrap Capto adhere ImpRes	5 × 1 ml	17-3715-10

Related products	Quantity	Code No.
Capto adhere ImpRes	25 ml	17-3715-01
	100 ml	17-3715-02
	1 l [1]	17-3715-03
	5 l [1]	17-3715-04
	10 l	17-3715-05
HiTrap Desalting	1 × 5 ml	29-0486-84
	5 × 5 ml	17-1408-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5078-01
	4 × 53 ml	17-5087-02

[1] Process-scale quantities are available. Please contact your local representative.

Accessories HiScreen	Quantity	Code No.
HiTrap/HiPrep, 1/16" male connector for ÄKTA *(For connection of columns with 1/16" fittings to ÄKTA)*	8	28-4010-81
Union 1/16" male/1/16" male with 0.5 mm i.d. *(For connecting two columns with 1/16" fittings in series)*	2	18-1120-93
Fingertight stop plug, 1/16" [1] *(For sealing a HiScreen column)*	5	11-0003-55

[1] One fingertight stop plug is connected to the inlet and the outlet of each HiScreen column at delivery.

Accessories HiTrap	Quantity	Code No
1/16" male/luer female *(For connection of syringe to top of HiTrap column)*	2	18-1112-51
Tubing connector flangeless/M6 female *(For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(For connection to original FPLC System through top of HiTrap column)*	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTA	8	28-4010-81
Stop plug female, 1/16" (For sealing bottom of HiTrap column)	5	11-0004-64
Fingertight stop plug, 1/16"	5	11-0003-55

Related literature	Code No.
Data file, Capto adhere ImpRes	29-0344-97
Application note, MAb polishing in Bind & Elute mode using Capto adhere ImpRes	29-0273-38

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