29091598 Superose™ 6 Increase 3.2/300

Read these instructions carefully before using the column.

Intended use

Superose 6 Increase 3.2/300 column is 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 product in a safe way, refer to the Safety Data Sheet.

Quick information

Superose 6 Increase 3.2/300 is a pre-packed high performance glass column. It is intended for sensitive and high resolving gel filtration of proteins, peptides, polynucleotides and other biomolecules in the micro preparative scale.

The column is supplied with two fingertight connectors 1/16" male for connection to ÄKTA™ or other systems. The column cannot be opened or refilled.

Table 1. Resin data

Matrix	Composite of cross-linked agarose
Particle size, d_{50V}[1]	~ 8.6 µm
Exclusion limit (M_r)	Approx. 4×10^7
Fractionation range	
globular proteins (M_r)	5000 to 5×10^6
dextrans (M_p)	1000 to 3×10^5
pH stability range	
operational[2]	3 to 12
CIP[3]	1 to 14
Temperature	
operational	4°C to 40°C
storage	4°C to 30°C

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Table 2. Column data

Bed dimensions (mm)	3.2 x 300
Approximate bed volume (mL)	2.4
Column efficiency (N/m)	> 48 000
Typical pressure drop over packed bed[1]	2.0 MPa[2] (20 bar, 290 psi)
Column hardware pressure limit	5 MPa (50 bar, 725 psi)

[1] Determine the limit according to section *Setting column pressure limits.*
[2] At maximum flow rate at 25°C in water.

Table 3. Flow rate limits and recommendations

Temperature		Flow rate (mL/min)
20°C to 25°C	Maximum flow rate, water	0.15
	Recommended flow rate, water	0.04
	Maximum flow rate, 20% ethanol	0.07
	Maximum flow rate, 10% glycerol	0.07
4°C to 8°C	Maximum flow rate, water	0.07
	Maximum flow rate, 20% ethanol	0.03
	Maximum flow rate, 10% glycerol	0.03

Note: *Most water-based buffers can be considered to be similar to water, for example phosphate and Tris buffers.*

Note: *When running viscous samples (for example containing glycerol) it is important to lower the flow rate, see recommendations above.*

First time use

Before connecting the column to a chromatography system, make sure there is no air in the tubing and valves. Remove the storage/shipping device and the stop plug from the column, see section *Delivery/storage*. Make sure that the column inlet is filled with liquid and connect it drop-to-drop to the system. For maximum resolution on the column, minimize dead volumes between the injection valve and the column as well as between the column outlet and the detector.

Prepare the column for first-time use as follows:

a) Equilibrate with at least 2 column volumes (CV) of room tempered water at a flow rate of 0.04 mL/min. Be aware to lower the flow rate if run at lower temperature.

b) Determine the column specific maximum pressure according to section *Setting column pressure limits.*

c) Equilibrate with at least 2 CV eluent at a flow rate of 0.04 mL/min.

d) It is recommended to perform a column performance control for future comparisons. See section *Column performance control.*

NOTICE
Make sure not to exceed the pressure limits of the column. This is particularly important when working at low temperatures, like in a cold room, or when the column is used with 20% ethanol or other viscous solutions. Decrease the flow rate according to Table 3. Set pressure limits according to section *Setting column pressure limits.*

Setting column pressure limits

There are two pressure limits to consider when running the column, the pressure drop over the packed bed and the column hardware pressure limit. The pressure drop over the packed bed differ for each column and the limit has to be individually set **as the column is packed to withstand the maximum flow rate**. Note that it might be different compared to the value noted in Table 2.

Exceeding any pressure limit may lead to collapse of the gel bed or damage to the column hardware. Increased pressure is for example generated when running/using one or a combination of the following parameters:

- Eluent or sample with high viscosity compared to water. This includes 20% ethanol.
- Low temperature compared to room temperature
- Modifications to the flow path, for example changing to thinner/ longer tubing

For optimal functionality it is important to know the pressure drops over different parts of your system and how they affect the column. All ÄKTA chromatography systems measure pressure at the system pump, $\mathbf{p_{pump}}$ (see Fig. 1). Some systems have additional pressure sensors located before and after the column, $\mathbf{p_{pre\text{-}cp}}$ and $\mathbf{p_{post\text{-}cp}}$.

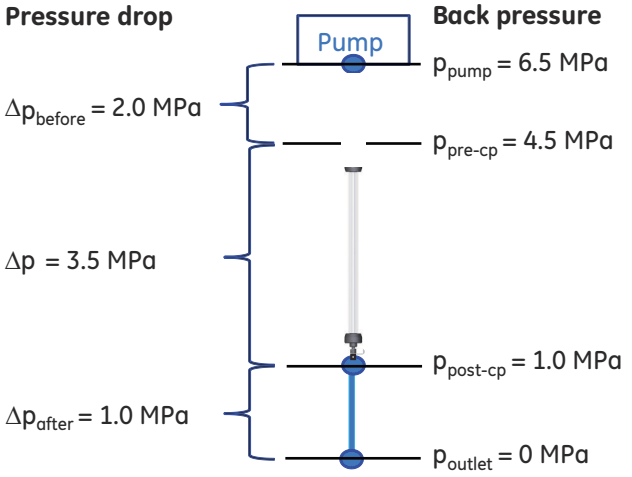


Fig 1. Example of the pressure in different parts of a system during run of a column.

- $\Delta\mathbf{p_{before}}$ does not affect the column.
- The pressure on the column hardware is the sum of $\Delta\mathbf{p_{after}}$ and $\Delta\mathbf{p}$. Do not exceed the column hardware limit!
- $\Delta\mathbf{p}$, is individual for each column and needs to be determined.

For more information, refer to the *ÄKTA laboratory-scale Chromatography Systems Instrument Management Handbook*.

How to set pressure limit for ÄKTA explorer, ÄKTA purifier, ÄKTA micro, and other systems with a pressure sensor in the pump

Determination of column specific pressure drop over the packed bed, $\Delta\mathbf{p}$ (see Fig. 1):

1 $\Delta\mathbf{p_{before}}$ is measured in absence of the column. Run the pump at maximum flow rate of the column in water and at the temperature for the experimental conditions. For exact values, see Table 3. Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\Delta\mathbf{p_{before}}$.

2 Check that the *Pressure Alarm* in software is set to the same as the Column hardware pressure limit.

3 Connect the column to the system. Let the flow drip from the column outlet. The column should be equilibrated in water and at the temperature for the experimental conditions. Run the pump at the same flow rate as in step 1. Note the pressure value.

4 $\Delta\mathbf{p}$ is calculated as the pressure value in step 3 minus $\Delta\mathbf{p_{before}}$. The $\Delta\mathbf{p}$ value will be used in step 8 below.

This $\Delta\mathbf{p}$ should not be exceeded at any temperature or using any liquid.

Setting pressure limit in method at your experimental conditions (intended system setup, flow rate, temperature and eluent):

5 $\Delta\mathbf{p_{before}}$ is measured in absence of the column. Run the pump at your intended flow rate. Let the flow drip from the tubing that will later be connected to the column. Note the pressure as $\Delta\mathbf{p_{before}}$.

6 Instead of the column, connect a piece of tubing[1] to the system. Run the pump at the same conditions as in step 5. Note the pressure value as the total system pressure.

7 $\Delta\mathbf{p_{after}}$ is calculated as the total system pressure value noted in step 6 minus $\Delta\mathbf{p_{before}}$, noted in step 5.

8 Calculate $\Delta\mathbf{p} + \Delta\mathbf{p_{after}} + \Delta\mathbf{p_{before}}$

- If this value is lower than the Column hardware pressure limit (see Table 2), set the pressure limit in your method as $\Delta\mathbf{p} + \Delta\mathbf{p_{after}} + \Delta\mathbf{p_{before}}$.
- If $\Delta\mathbf{p} + \Delta\mathbf{p_{after}}$ exceeds the column hardware pressure limit, reduce the flow rate or $\Delta\mathbf{p_{after}}$. Repeat step 5-8.

You can now start your experiment!

[1] Avoid thin and/or long tubing that will give back pressure.

How to set pressure limit for ÄKTA pure, without a column valve or with Column Valve V9-Cs (1 column)

$\mathbf{p_{pre\text{-}c}}$ (see Fig. 1) is automatically monitored by the system. This is the pressure signal to use in the following instruction. Do not use the *System pressure* signal. Note that the measured values include the tubing used to connect the column to the instrument.

Determination of column specific pressure drop over the packed bed ($\Delta\mathbf{p}$):

1 Check that the *Alarm pre column pressure* in software is set to the same as the Column hardware pressure limit (see Table 2).

2 Connect the column to the system. Let the flow drip from the column outlet. The column should be equilibrated in water and at the temperature for the experimental conditions. Run the pump at maximum flow rate of the column in water and at the temperature for the experimental conditions. For exact values, see Table 3.

Note the pressure value.
The now measured $\mathbf{p_{pre\text{-}c}}$ value is the maximum pressure over the packed bed, $\Delta\mathbf{p}$ (*DeltaC pressure*).

3 This $\Delta\mathbf{p}$ value should not be exceeded at any temperature or with any liquid!

Setting pressure limit in method at your experimental conditions (intended system setup, flow rate, temperature and eluent):

4 $\Delta\mathbf{p_{after}}$ is measured in absence of the column. Run the pump at your intended flow rate.

5 Instead of the column, connect a piece of tubing[1] to the system, or bypass the column if connected to a valve. Run the pump at your intended flow rate.
The now measured value is $\Delta\mathbf{p_{after}}$.

6 Calculate $\Delta\mathbf{p} + \Delta\mathbf{p_{after}}$.

- If this value is lower than the Column hardware pressure limit (see Table 2), set the pressure limit in your method, *Alarm pre column pressure*, as $\Delta\mathbf{p} + \Delta\mathbf{p_{after}}$
- If $\Delta\mathbf{p} + \Delta\mathbf{p_{after}}$ exceeds the column hardware pressure limit, reduce the flow rate or $\Delta\mathbf{p_{after}}$.
  Repeat step 4-6.

You can now start your experiment!

Column Valve V9-C for ÄKTA pure and ÄKTA avant

Note: *It is not recommended to use Valve V9-C due to large dead volume.*

Column performance control

In order to detect any changes in column performance, it is very important that you make an initial test with your particular system configuration. Note that the contribution from dead volumes in the instrument to band broadening will vary depending on system set-up and will influence column efficiency, thus the obtained efficiency on your system might be lower compared to the specifications in Table 2.

Column efficiency test

Column efficiency, expressed as the number of theoretical plates per meter, N/m, is calculated using the following equation:

$$N/m = 5.54 \times (V_R / W_h)^2 / L)$$

where

N/m	=	number of theoretical plates/meter
V_R	=	volume eluted from the start of sample application to the peak maximum
W_h	=	peak width measured as the width of the recorded peak at half of the peak height
L	=	bed height (m)

Check the performance of the column using the following procedure:

Sample:	10 µL 2% acetone (20 mg/mL) in buffer or water
Eluent:	Buffer or water
Flow rate:	0.1 mL/min, room temperature
Detection:	280 nm

Function test

As an alternative to the above efficiency test, check the column performance by running the function test shown in Fig. 2.

Sample:	1. Thyroglobulin (M_r 669 000) 3 mg/mL
	2. Ferritin (M_r 440 000) 0.3 mg/mL
	3. Aldolase (M_r 158 000) 3 mg/mL
	4. Ovalbumin (M_r 44 000) 3 mg/mL
	5. Ribonuclease A (M_r 13 700) 3 mg/mL
	6. Aprotinin (M_r 6 500) 1 mg/mL
Sample volume:	10 µL
Eluent:	0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4
Flow rate:	0.04 mL/min, room temperature
Detection:	280 nm

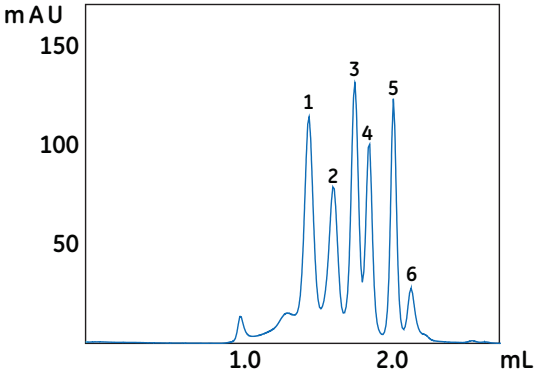


Fig 2. Typical chromatogram from a function test of Superose 6 Increase 3.2/300 using ÄKTAmicro.

[1] Avoid thin and/or long tubing that will give back pressure.

Try these conditions first

Eluent:	0.01 M phosphate buffer, 0.14 M NaCl, pH 7.4.
Flow rate: (room temperature)	0.04 mL/min
Sample volume:	10 µL

Equilibration is not necessary between runs with the same eluent buffer. Read the section *Optimization* for information on how to optimize a separation.

Sample recommendations

Molecular weight (M_r):	5000 to 5 × 10^6
Protein concentration:	Up to 50 mg/mL, for higher resolution below 10 mg/mL.
Sample volume:	4 to 50 µL
Preparation:	Dissolve the sample in eluent, filter through a 0.22 µm filter or centrifuge at 10 000 g for 10 min.

Note: *High sample viscosity (high protein concentration or additives) can cause instability of the separation and the back pressure might increase. Dilute sample or decrease flow rate during sample application.*

System recommendations

The small bed volume of the 3.2/300 column makes it sensitive to dead volumes in the system. It is recommended to use systems like ÄKTAmicro, ÄKTApurifier 10, ÄKTA pure 25, and HPLC systems. Use short, narrow capillaries and avoid all unnecessary components in the flow path. Valve V9C (5 columns) is not recommended in the flow path due to large internal volume. For optimal configuration of ÄKTA pure 25 see Cue Cards in the Literature list in Section *Ordering Information*.

ÄKTA start is not compatible with Superose 6 Increase columns due to too low maximum operating pressure.

ÄKTA avant is not recommended due to non-optimal dead volumes.

Superose 6 Increase 3.2/300 on different systems

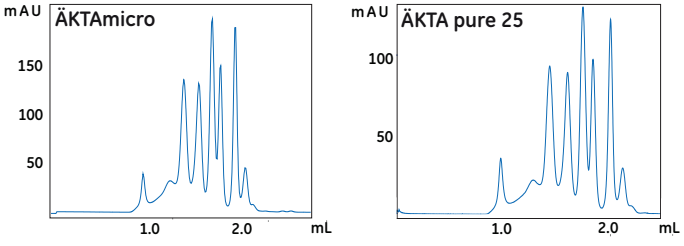


Fig 3. Comparison of protein separation on Superose 6 Increase 3.2/300 on different systems. Due to different UV cells for the systems, mAU scales differ

Delivery/storage

The column is delivered with a storage/shipping device that prevents it from drying out. The column is equilibrated with degassed 20% ethanol.

If the column is to be stored for more than 2 days after use, wash the column with 2 CV water and then equilibrate with at least 2 CV 20% ethanol.

Note: *Use a lower flow rate for 20% ethanol. See Table 3.*

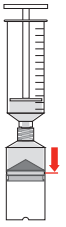
We recommend that you connect the storage/shipping device according to section *How to connect the storage/shipping device* for long term storage.

How to remove the storage/shipping device

1 Push down the spring-loaded cap.

2 Remove the locking pin.

3 Release the cap and unscrew the device.



How to refill the storage/shipping device

1 Connect a syringe or pump to the storage/shipping device and fill with 20% ethanol over the mark on the tube. Remove the syringe or connection to the pump.

2 Tap out air bubbles and push the plunger to the mark on the device.

How to connect the storage/shipping device

1 Fill the column inlet and luer connector with 20% ethanol and connect the filled storage/shipping device drop-to-drop to the top of the column.

2 Mount the spring-loaded cap (2) and secure it with the locking pin (3).

Choice of eluent

Select an eluent that ensures the sample is fully soluble. Also try to choose an eluent that will simplify downstream applications. For example, if the proteins/peptides are to be lyophilized, a volatile eluent is necessary. Since ionic interactions can occur with both acidic and basic proteins at very low salt concentrations, a recommended buffer is 0.01 to 0.05 M sodium phosphate, with additional 0.15 to 0.3 M NaCl, pH 7.4. Table 4 lists some useful eluent compositions.

Table 4. Useful eluent compositions

pH	Buffer/eluent	Properties/application examples
5.0	0.1 M ammonium acetate	Good solubility for some enzymes, e.g., cellulases. Volatile.
6.8	0.2 M sodium phosphate	Suitable for some antibody separations.
7.2	0.05 M phosphate + 0.15 M NaCl	Physiological conditions.
7.8	0.15 M ammonium hydrogen carbonate	Suitable for some DNA and protein separations. Volatile. Should be used fresh.
8.0	0.1 M Tris-HCl, 0.001 M EDTA	Very good solubility for DNA and RNA.
8.6	6 M guanidine hydrochloride in 0.05 M Tris-HCl	Good UV-transparency. Suitable if there is a need to purify proteins under denaturing conditions.
11.5	0.05 M NaOH	Good solubility for some compounds.

Buffer additives	Properties/application examples
Up to 8 M urea (pH<7)	Good solubility for many components. Biological activity can be maintained at lower urea contents. Certain risk for carbamylation of proteins.
6 M guanidine hydrochloride	Molecular weight determinations of subunits.
0.1% SDS, Tween™ or similar	Good solubility for some proteins, e.g., membrane proteins. Make sure you equilibrate completely with the detergent solution.
0.2 M arginine	Decreases tendency of aggregation.

Buffers and solvent resistance

De-gas and filter all solutions through a 0.22 µm filter. Install an on-line filter before the injection valve.

Note: *Buffers and solvents with increased viscosity will affect the back pressure. Reduce the flow rate if necessary. See Table 3.*

Long term use

Long term use refers to use where the resin is stable over a long period of time without adverse side effects on its chromatographic performance.

- All commonly used aqueous buffers, pH 3 to 12
- Urea, up to 8 M
- Ionic and non-ionic detergents, e.g., 1% SDS
- Guanidine hydrochloride, up to 6 M
- Isopropanol, up to 5%
- Methanol, up to 10%
- Sodium hydroxide, up to 0.5 M
- Dithiothreitol, up to 5 mM

Short term use

Short term use refers to the use during regeneration, cleaning-in-place, and sanitization procedures.

- Acetonitrile, up to 30%
- Sodium hydroxide, up to 1 M
- Ethanol, up to 70%
- Methanol, up to 100%
- Acetic acid, up to 1 M
- Isopropanol, up to 30%
- Hydrochloric acid, up to 0.1 M
- Trifluoroacetic acid, up to 10%
- Formic acid, up to 70%

Avoid:

- Oxidizing agents
- Unfiltered solutions

Optimization

If your results are unsatisfactory, consider the following actions.

Flow rate

Action: Decrease the flow rate.

Effect: Improves resolution for high molecular weight components. The resolution for small components may be decreased.

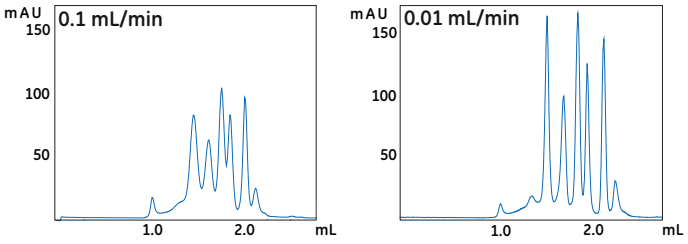


Fig 4. Comparison of protein separation on Superose 6 Increase 3.2/300 at different flow rates.

Sample volume

Action: Decrease the sample volume.

Effect: Improves resolution.

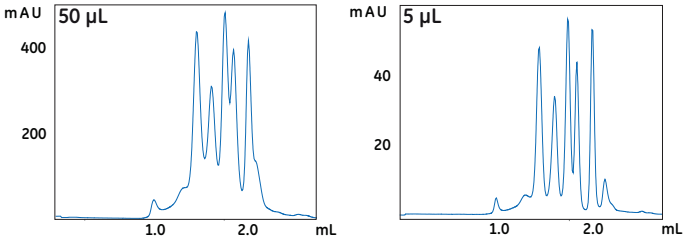


Fig 5. Comparison of protein separation on Superose 6 Increase 3.2/300 using different sample volumes.

System dead volumes

Action: Decrease system dead volumes.

Effect: Improves resolution.

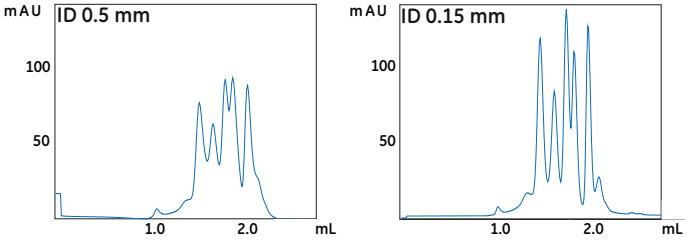


Fig 6. Comparison of protein separation on Superose 6 Increase 3.2/300 using different diameters of a 32 cm long capillary connected to the column.

For more information, refer to the handbook *Gel filtration, Principles & Methods*.

Cleaning-in-place (CIP)

Perform the following regular cleaning cycle after 10 to 20 separation cycles. Increasing pressure drop over the packed bed indicate that the column needs to be cleaned.

Note: *When performing CIP, reversed flow is recommended.*

Regular cleaning

1 Wash the column with 1 CV 0.5 M sodium hydroxide alternatively 0.5 M acetic acid at a flow rate of 0.02 mL/min.

2 Immediately rinse the column with 1 CV water followed by at least 2 CV eluent at a flow rate of 0.02 mL/min.

Before the next run, equilibrate the column until the UV baseline and pH are stable. Check that the column performance has been restored according to section *Column performance control*.

More rigorous cleaning

- Depending on the nature of the contaminants, the cleaning solutions in section *Buffers and solvent resistance* may be used. Always rinse with at least 2 CV water after any of the cleaning solutions have been used.
- If column performance is not restored, wash the column with 3 CV 0.5 M arginine. Rinse with at least 2 CV water.
- If column performance is still not restored, inject a solution of 1 mg/mL pepsin in 0.1 M acetic acid containing 0.5 M NaCl and leave overnight at room temperature or one hour at 37°C. After enzymatic treatment, clean the column according to the procedure described in the section *Regular cleaning*.

Troubleshooting

Symptom	Remedy
Increased back-pressure over the column and/or loss of resolution	Confirm that the column is the cause (see below). If so, clean it according to the procedure described in section *Cleaning-in-place (CIP)*. To confirm that the high back-pressure in the system is caused by the column, disconnect one piece of equipment at a time (starting at the fraction collector) with the pumps running. Check the pressure reading after each piece has been disconnected to determine the source of the back-pressure.
Air in the column	Note that small amounts of air will normally not affect the performance of the column. Run 3 to 4 CV well de-gassed eluent in an upflow direction at a flow rate of 0.04 mL/min at room temperature.
Issues with removing shipping/storage device, stopper or connector	Carefully tighten the black end cap in both ends of the column by hand.
Loose end cap	Do not use the column in a system if the black end caps are loose. This is because the liquid could leak into the space between the inner column and the outer shell. Carefully tighten the black end caps by hand.

Ordering information

Product	Quantity	Product code
Superose 6 Increase 3.2/300	1	29091598

Related products

Product	Quantity	Product code
Superose 6 Increase 10/300 GL	1	29091596
Superose 6 Increase 5/150 GL	1	29091597
Superdex 30 Increase 10/300 G	1	29219757
Superdex 30 Increase 3.2/300	1	29219758
Superdex 75 Increase 10/300 GL	1	29148721
Superdex 75 Increase 5/150 GL	1	29148722
Superdex 75 Increase 3.2/300	1	29148723
Superdex™ 200 Increase 3.2/300	1	28990946
Superdex 200 Increase 5/150 GL	1	28990945
Superdex 200 Increase 10/300 GL	1	28990944
Gel filtration LMW Calibration Kit	1	28403841
Gel filtration HMW Calibration Kit	1	28403842

Accessories

Product	Quantity	Product code
Fingertight connector, 1/16" male	10	18111255
Tricorn™ storage/shipping device	1	18117643

Literature

Document	Product code
Size Exclusion Chromatography, Principles & Methods	18102218
ÄKTA laboratory-scale Chromatography Systems Instrument Management Handbook	29010831
Procedure: Maintenance and cleaning of size exclusion chromatography columns	29140760
Cue Cards: Optimal configuration of ÄKTA pure 25 for small scale SEC	29181181

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