TA-SP XL

High-Capacity Strong Cation Exchange Chromatography Resin

Product Manual

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1. Product Introduction

Ion exchange chromatography (IEX) is a very effective method for the separation and purification of biomolecules, which mainly relies on the interaction between positive and negative charges, and utilizes the differences in the nature and amount of charges carried by different biomolecules under specific conditions to carry out the separation, and it is characterized by high loading capacity, good resolution, controllable conditions, and easy to be amplified. It has been widely used in medicine, chemical industry, metallurgy, food and other fields. Ion exchange media is composed of three parts: (1) crosslinked mesh framework, the framework has a porous, hydrophilic, good chemical stability; (2) fixed on the framework of the functional group, it is the charge group, determines the nature of the ion exchange chromatography media; (3) and the functional group of the ion with an opposite charge (can be called the balance of the ion), the ion can be reversible with the combination of the functional group.

TA-XL series media are coupled with linear dextran molecules on a highly cross-linked agarose base frame, and then coupled with various functional groups on the dextran molecules. This structure reduces the site-barrier effect between biomolecules, and can greatly improve the loading of target molecules.

TA-SP XL is a strong cation exchange medium that can capture biomolecules directly from the feed solution, which can meet the needs of downstream separation processes and is easy for large-scale production.

Table 1 TA-SP XL technical parameters

Appearance	White paste, layered on placement
Framework	6% highly cross-linked agarose with dextran chains
Functional groups	-$(CH_2)_3SO_3^-$(sulfopropyl)
Particle size distribution range	45~165μm(average 90μm)
Ion loading	180~250μmol H^+/mL filling medium
Pressure resistance	0.3 MPa
Operating pH range	4~13
Chemical stability	Common aqueous phase solution, 1M NaOH, 6M guanidine hydrochloride, 30% isopropanol, 70% ethanol
pH Stability	3~14 (CIP); 4~13 (working)
Temperature tolerance	Use temperature 4~40℃, can not freeze
Storage	2~30℃, 20% ethanol or 2% benzyl alcohol, 0.2M NaAc
Recommended Flow Rate	60~300cm/h
Precautions	Avoid contact with oxidizing agents, cationic detergents. Do not expose to pH <4 for a long time (1 week, 20°C).

2. Methods of use

2.1 Chromatography column loading

Note: It is best to equilibrate the media suspension to room temperature before loading the column.

- Calculate the amount of TA-SP XL needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.15 (i.e., compression ratio of approximately 1.15)

  Volume of media suspension required = volume of settling media ÷ concentration of media suspension.

Note: The concentration of Truking Microsphere's original packaging media suspension is 80%, for non-original concentration of media suspension, customers can calculate the required volume according to the actual concentration of media suspension.

- Replacement solution: pour the gel suspension into a Brinell's funnel, draw off the liquid and wash it with about 3 times the volume of the loading solution (purified water), (when the volume is relatively large or the conditions are not available, you can also use to wait for the gel to be delaminated to draw off the upper layer of solution, and then add an appropriate amount of loading solution stirring and wait for the delamination to be withdrawn, and then repeat the method of replacing the media solution for 3 times)
- Gel suspension preparation: In order to get a better column loading effect, the concentration of the gel suspension should be between 50% and 75%, i.e., the volume of the sinking gel is one-half to three-quarters of the total volume, stir well and set aside.
- Take a cleaned TK-EC chromatography column (the diameter of TK-EC series chromatography columns ranges from 1cm to 45cm in various specifications to meet the different sizes of chromatography applications), drain the membrane air bubbles at the bottom of the column and keep about 1cm high liquid column at the bottom of the column, and adjust the column so that it is perpendicular to the ground.
- Pour the stirred gel suspension slowly into the chromatography column one at a time (using a column loader if necessary), taking care not to introduce air bubbles.
- Connect the upper column head to the chromatography system or peristaltic pump, drain the air bubbles under the screen of the upper column head (for the chromatography column with diameter less than 20cm, you can use the peristaltic pump or the earwash ball to suck out the air bubbles under the screen after turning the column head upwards), put the column head into the chromatography column, shake the column head so that the air bubbles can be discharged from the edge of the column head, and then screw the sealing knob tightly. (For the chromatography column with diameter >30cm, do not tighten the sealing ring too much, press down the head of the shaft to let the liquid inside the column back out through the column head to discharge the air bubbles inside the column head, and then tighten the sealing knob).
- Set the flow rate (for the loading height of 15~20cm TA-SP XL can be set to 300cm/h), start the pump pressure until the gel surface is stable, if the pressure exceeds 0.3MPa during the loading process, the flow rate needs to be reduced appropriately. Mark the height of the column bed when it is stable.
- Remove the column loader (if there is one), lower the column head to the position of about 0.5cm on the gel surface, continue to press the column according to the flow rate of 480cm/h until the gel surface is clear and stable, mark the position of the gel surface.
- Stop the pump, open the valve/plug on the column head, close the valve/plug at the bottom of the column, slightly relax the sealing ring of the column head, lower the column head to about 0.3cm below the marked position, tighten the sealing ring of the column head, close the valve/plug at the column head, and the column loading is completed.

2.2 Column Effectiveness Evaluation

Column efficiency can be determined by using acetone as indicator or NaCl as indicator, and the indicator solution and mobile phase are prepared according to the following table.

Table 2: Column efficiency determination methods

Methods	Acetone Method for Column Efficacy	Column Efficacy by NaCl Method
Sample	1.0% (v/v) acetone in water	0.8M NaCl (dissolved in water)
Sample volume	1.0% column volume	1.0% column volume
Mobile phase	Water	0.4M NaCl aqueous solution
Flow rate	30 cm/h	30 cm/h
Detection Data	UV 280 nm	Conductivity

2.3 Calculating Column Effect

Theoretical plate height (HETP), theoretical number of plates (N) and asymmetry factor (As) were calculated from the UV or conductivity curves with the following equations:

HETP=L/N

$N=5.54(V_R/W_h)^2$

Where: V_R = retained volume

W_h=half peak width

L=column height

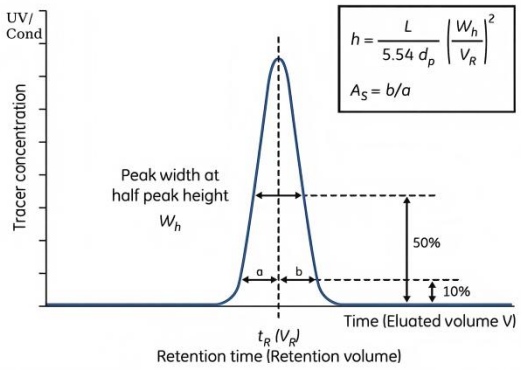
N=theoretical plate number

The units of V_R and W_h should be the same;

As=b/a

Where: a= first half peak width at 10% peak height

b= second half peak width at 10% peak height



2.4 Evaluation of results

$h=HETP/d_{50v}$

d_{50v} = median particle size volume distribution (cm)

The h-value calculated by the above formula is less than 3, and the asymmetry factor is 0.8~1.8 then it is judged to be qualified. For unsatisfactory column efficiency the reason needs to be analyzed and the column reloaded.

2.5 Chromatographic methods

- **Buffer selection:** buffer salts whose buffer groups do not interact with the medium should be selected; low salt (less than 5ms/cm) and low pH (usually 1 pH unit below the isoelectric point of the target) buffers are preferred for equilibrium buffers to favor the binding of the target, and the stability of the target in the buffer needs to be considered.The elution buffer is usually a buffer with a high concentration of salt (e.g. 1M NaCl) added to the equilibration buffer or a high pH elution.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous membrane and the pH and conductivity of the sample adjusted to match that of the equilibration buffer prior to sampling (the pH and conductivity of the sample can be adjusted by dilution, ultrafiltration, and replacement of the buffer with TD-G25).
- **Equilibration:** The pH and conductivity of the buffer used to flush the chromatography column to the outlet

with equilibration buffer is essentially the same as that of the equilibration buffer, and usually requires 3 to 5 times the column bed volume.

- **Sampling:** The volume of the sample is determined according to the content of the substance in the sample and the binding load of TA-SP XL.
- **Rinse:** Rinse the column with equilibration buffer until the UV absorption is close to baseline.
- **Elution:** A linear gradient or step gradient can be used to increase the elution strength in the eluent to elute substances with different binding strengths from the chromatography column and collect different fractions to detect where the target is located.
- **Regeneration:** Flush the chromatography column with a salt containing a high concentration (e.g. 2M NaCl).
- **Re-equilibration:** Rinse with the equilibration buffer, and when the pH and conductivity are essentially the same as the equilibration buffer, a second sample can be taken, and so on.

3. Cleaning and regeneration

As the number of times the chromatography medium is used increases, the accumulation of contaminants on the chromatography column also increases. Regular in-situ cleaning prevents the accumulation of contaminants and maintains a stable working condition. Determine the frequency of in-situ cleaning according to the degree of contamination of the media (if contamination is severe, it is recommended that in-situ cleaning be performed after each use to ensure reproducibility of results).

Recommended cleaning conditions for different types of impurities and contaminants are as follows:

- Removal of tightly bound proteins: Wash with 2~3 column volumes of 2M NaCl.
- For the removal of strongly hydrophobic proteins and precipitated proteins: first wash with 2-3 column volumes of 1M NaOH, then immediately rinse with 5-10 column volumes of pure water.
- Removal of lipoproteins and lipids: Wash with 5-10 column volumes of 70% ethanol or 30% isopropanol, followed by a rinse with 5-10 column volumes of pure water.
- Cleaning can also be carried out by combining the above two cleaning conditions, i.e., cleaning with a 30% isopropanol solution containing 1M NaOH.

Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate can be selected from 30-60cm/h during bit cleaning; reverse cleaning can be used when the blockage is serious.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solution containing 0.2M sodium acetate does not have sterilizing and de-pyrogenic effects, it is recommended that TA-SP XL can be treated with 1M NaOH for 0.5~1h before and during use to reduce the risk of microbial contamination.

TA-SP XL is sold in 20% ethanol or 2% benzyl alcohol containing 0.2M sodium acetate as a preservation solution. After use, TA-SP XL should be stored in 20% ethanol containing 0.2M sodium acetate in a closed container at 2-30°C. To prevent evaporation of ethanol and microbial growth, it is recommended that the preservative be replaced with a fresh solution every 3 months.

5. Destruction and recycling

Since TA-SP XL is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm
TA-SP XL	Y2075	25mL
	Y2076	100mL
	Y2077	500mL
	Y2078	1L
	Y2079	5L
	Y2080	10L
	Y2081	20L