TH-Protein A

Affinity Chromatography Resin

Product Manual

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Content

1. Product Introduction

TH-Protein A is a new type of affinity chromatography medium made by coupling alkali-resistant Protein A with a highly rigid agarose matrix through epoxy activation. Compared with traditional antibody affinity media, it has a more stable ligand, faster flow rate, and lower backpressure, and is suitable for the capture of monoclonal antibodies or Fc fusion proteins from high-volume cell cultures, and for the capture of monoclonal antibodies or Fc fusion proteins from peritoneal fluid or blood plasma.It is also suitable for capturing polyclonal antibodies from ascites or plasma.

The ligand of TH-Protein A is a recombinant protein A fragment obtained by fermentation of E. coli. In the upstream construction, base-resistant amino acids were used to replace non-base-resistant amino acids, and protease-sensitive amino acids were removed, which made the ligand have good base stability, and the fermentation of the ligand and the subsequent purification process used raw materials of no animal source.The medium can be cleaned in-situ with 0.1-0.5M NaOH, avoiding the use of expensive and corrosive cleaning reagents, which can effectively save costs and is easy to be used in industrialized mass production.

The medium has the following characteristics:

- High hardness, low backpressure, fast flow rate, suitable for mass production applications.
- High loading capacity, which can reduce the production cost.
- Resistant to alkali cleaning and has a long service life.

Table 1: TH-Protein A technical parameters

Appearance	White paste, layered on placement
Base frame	Highly rigid agarose
Average particle size	75μm
Functional group	Recombinant alkali-resistant Protein A (24kD)
Crosslinking mode	Epoxy chemical
Dynamic binding load	>55mg human IgG/mL medium
Chemical stability	TH-Protein A medium is stable in all commonly used buffers; resistant to 10mM HAc, 0.1M sodium citrate, 6M guanidine hydrochloride, 8M urea, 30% isopropanol wash, and can be stored for a long time in 20% ethanol.
Pressure resistance	0.5MPa
Pressure flow rate	300cm/h, <2bar, TK-EC 300/500, H=20cm
pH stability	3-12 (working); 2-13 (CIP, short-term); CIP: 0.1-0.5M NaOH
Storage	2~8°C, 20% ethanol or 2% benzyl alcohol
Recommended Flow Rate	60~300cm/h
Note	The load of the resin hardly changes within 30 days at room temperature, so please feel free to use our samples. However, long-term storage beyond 30 days is recommended at 2~8°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 TH-Protein A needed based on the volume of the chromatography columns

  Settling volume required = column volume x 1.10 (i.e., compression ratio of approximately 1.10)

  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.

- Media washing: Shake the media suspension well and measure the volume calculated by the above method, pour it into a funnel, draw off the liquid and wash it with about 3mL of column filling solution (20% ethanol + 0.4M NaCl)/mL of media, repeat the washing 3 times, each time when adding the washing solution, it is necessary to stir it with a glass rod or stirring stick in order to wash off the original preservation solution better.
- Preparation of Column Mounting Gel Suspension: Transfer the cleaned medium from the funnel to a beaker or other suitable container, add the column mounting solution until the concentration of the gel suspension is 45~55%, 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, taking care not to bring in 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).
- When the column height is 20cm, you can set the loading flow rate to 200cm/h, open the bottom valve/plug of the chromatography column, turn on the flow rate, and use the set flow rate to press until the gel surface is clear and stable, and mark the position when the gel surface is stable.
- Remove the column loader (if there is one), lower the column head to about 0.5 cm above the gel surface, continue to press the column at the flow rate above until the gel surface is clear and stable, and then mark the height of the column at which the gel surface is stable.
- 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, press down the rubber surface according to the compression ratio of 1.10, tighten the sealing ring of the column head, close the valve/plug at the column head, and the installation of the column 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

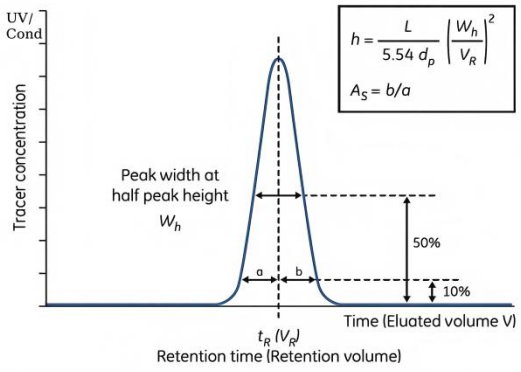
N=theoretical plate number

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:** a neutral buffer is usually used as the binding buffer (e.g., 50 mM PB, 0.15 M NaCl, pH 7.0-7.6), and a low pH buffer is used as the eluent (e.g., 0.1 M citric acid, pH 3.0-4.0), since the ability of Protein A to bind to IgG is dependent on the source of the antibody and its subtype (Table 3), high salt and high pH promote antibody and mediator binding and reduce nonspecific binding, and increasing pH neutralizes histidine residues relative to the base-resistant Protein A and IgG binding sites.The electrostatic repulsive effect of these residues impedes the affinity response.Increase salt concentration to reduce electrostatic repulsion and enhance hydrophobicity.Optimization of binding and washout conditions can be performed for different antibodies by varying the type and concentration of salt in the buffer as well as the pH.

  When optimizing the elution conditions, it is necessary to map out the highest pH for effective desorption to

avoid causing denaturation of unstable antibodies at too low a pH, but too high an elution pH may result in a loss of yield.

- **Flow rate:** According to the height of the column generally choose a linear flow rate of 60~300cm/h, the higher the height of the column the slower the flow rate.
- **Sample Preparation:** To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45 μm microporous filter membrane and the pH and conductivity of the sample adjusted to match the equilibrium buffer before loading, with the lower the flow rate the higher the dynamic binding load.
- **Equilibration:** Fully equilibrate the chromatography column with equilibration buffer, usually 3 to 5 column volumes are required.
- **Sampling:** The volume of the sample is determined according to the content of the substance in the sample and the binding load of TH-Protein A. Sampling is performed.
- **Cleaning:** Equilibrium buffer is used to reduce the UV absorption to a suitable value after the samples have been taken, and high salt or slightly lower pH can be added for cleaning if necessary, so as to clean down as many non-specifically adsorbed impurities as possible.
- **Elution:** low pH buffer is usually used for elution, the optimization of elution conditions can be determined by using a linear gradient of pH, usually a 10 column volume linear gradient from equilibrium to elution buffer (e.g.: 1M citric acid, pH 3.0) is used to determine the optimal pH of the eluent based on the location of the peak. if the antibody is unstable under acidic conditions, the eluent can be neutralized with an appropriate volume of neutralizing solution (e.g.:1.0 M Tris-HCl, pH 9.0) to neutralize.

Table 3 Comparison of affinity of Protein A for specific subtypes of monoclonal antibodies

Antibody	Affinity	Binding pH	Elution pH
Human			
IgG_1	Very high	6.0-7.0	3.5-4.5
IgG_2	Very high	6.0-7.0	3.5-4.5
IgG_3	Low-No	8.0-9.0	≤7.0
IgG_4	Low-High	7.0-8.0	3.0-6.0
Mice			
IgG_1	Low	8.0-9.0	4.5-6.0
IgG_{2a}	Medium	7.0-8.0	3.5-5.5
IgG_{2b}	High	≥7.0	3.0-4.0
IgG_3	Low-High	≥7.0	3.5-5.5

2.6 Lifetime verification

Sample: CHO cell antibody culture solution

Equilibration solution: 20mM PB, 150mM NaCl, pH= 7.4;

High salt elution solution: 20mM PB , 1M NaCl, pH= 7.4;

Low salt elution solution: 20mM NaAc, pH=5.0;

Elution solution: 20mM NaAc, pH=3.6;

Neutralization solution: 1M Tris-HCl, pH=9.0;

Regeneration solution: 1M HAc, pH=2.5;

Cleaning solution: 0. 1M NaOH, pH=12.9.

2.6.1 Curve fit

The chromatograms of 200 cycles are shown below after harmonizing the axes, with a good curve fit.

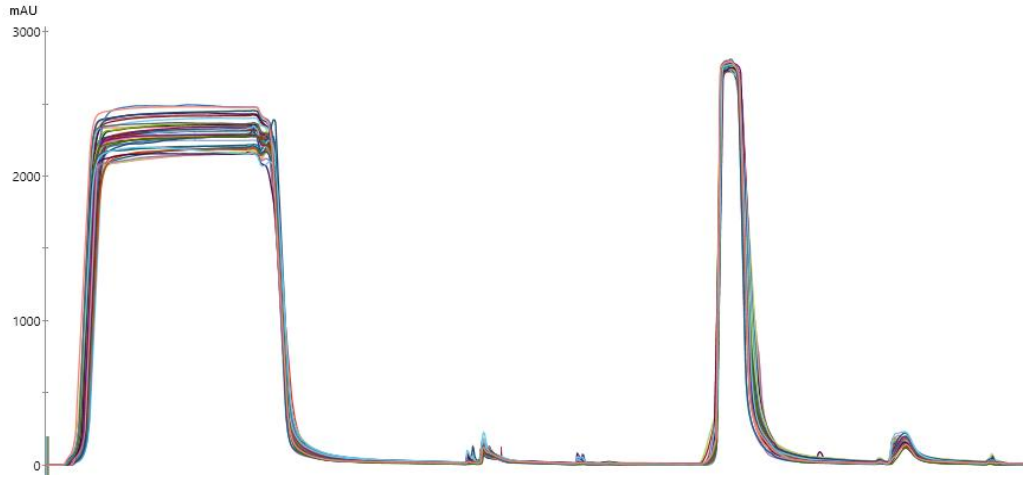


Figure 1 TH-Protein A 200cycle Chromatographic Fits

2.6.2 Dynamic binding capacity

The test results showed that the initial dynamic loading of TH-ProteinA was 72 mg/ml, and the dynamic loading was 57.63 mg/ml after 200 cycles, with a decrease in dynamic loading of less than 20%.

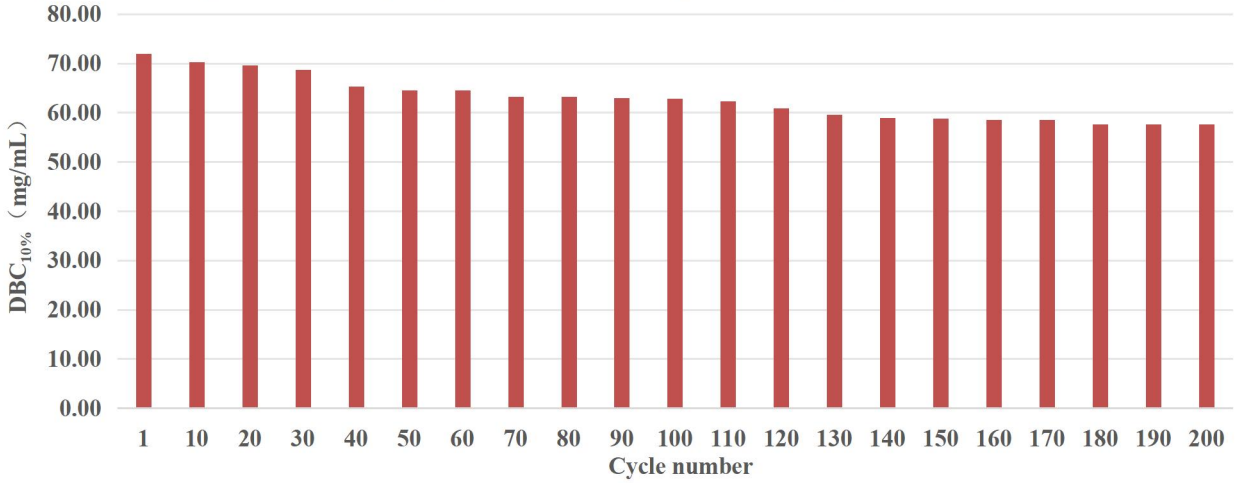


Fig. 2 TH-Protein A 200cycle $DBC_{10\%}$ change

2.6.3 Yield

The change in yield for 200 cycles is shown in the figure, and the yield remained at about 90% after 200 cycles.

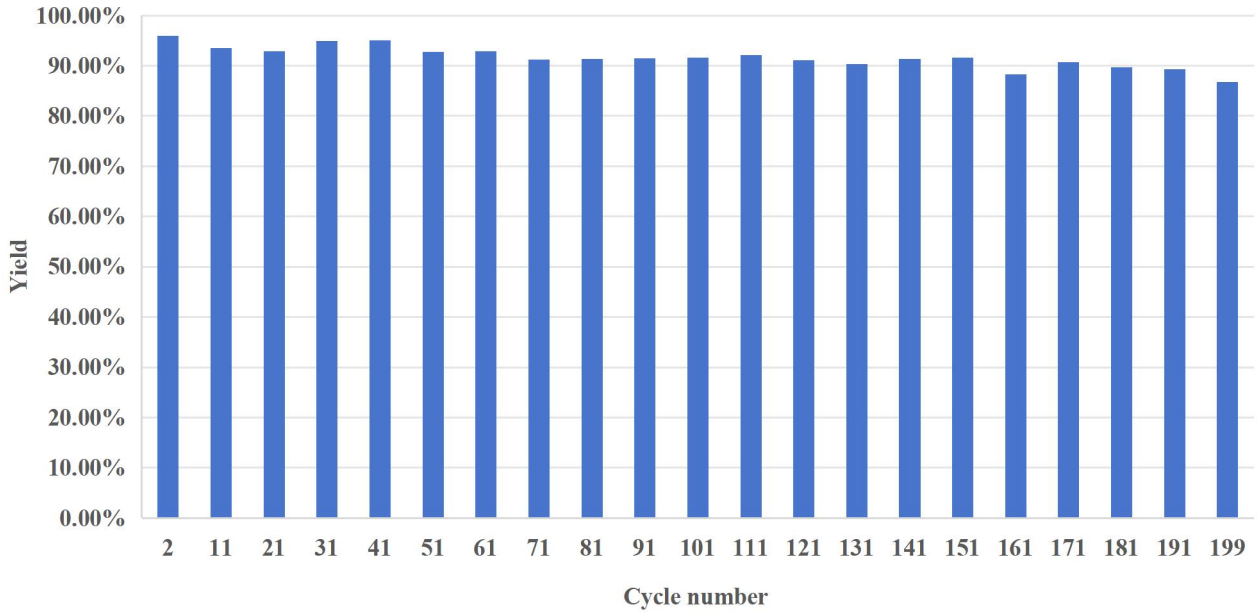


Figure 3 TH-ProteinA lifetime test elution sample yield assay data

2.6.4 Purity

The change in purity over 200 cycles is shown in the figure, and the purity of the eluted samples remained at about 98% after 199 cycles.

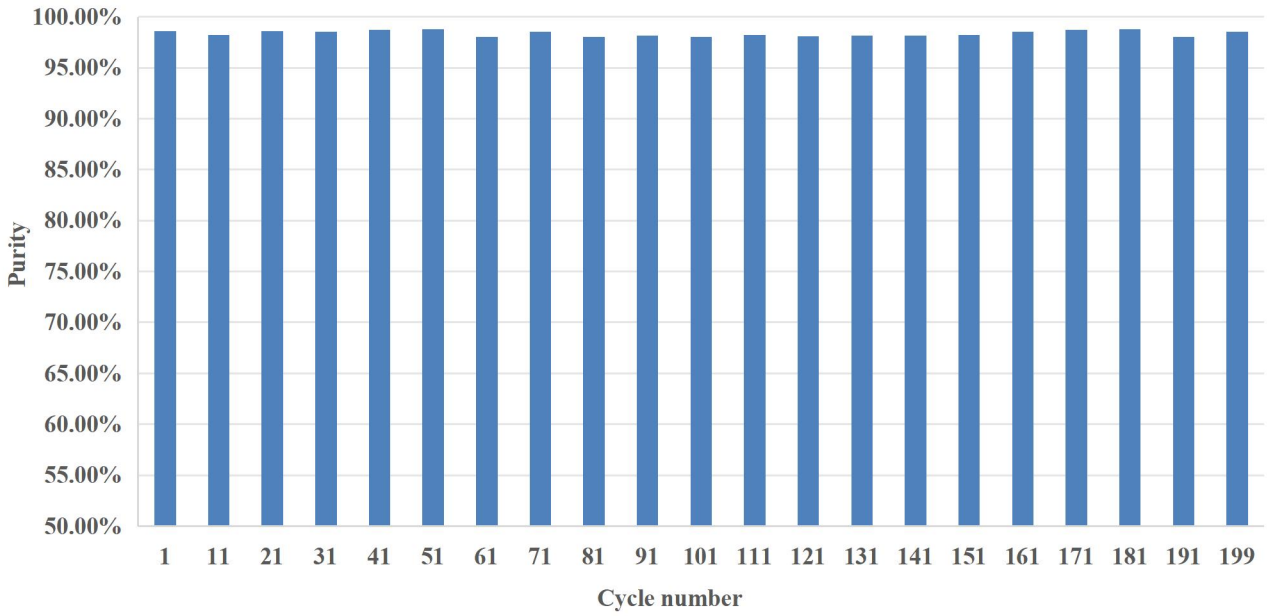


Figure 4 TH-ProteinA Lifetime Test Elution Sample Purity Assay Data

2.6.5 Delta Pressure

The sample pressure, elution pressure and CIP pressure for 200 cycles are shown in Fig. With the increase of the number of cycles, the Delta Pressure gradually becomes larger, but it always stays below 0.2 MPa, which indicates its better mechanical properties.

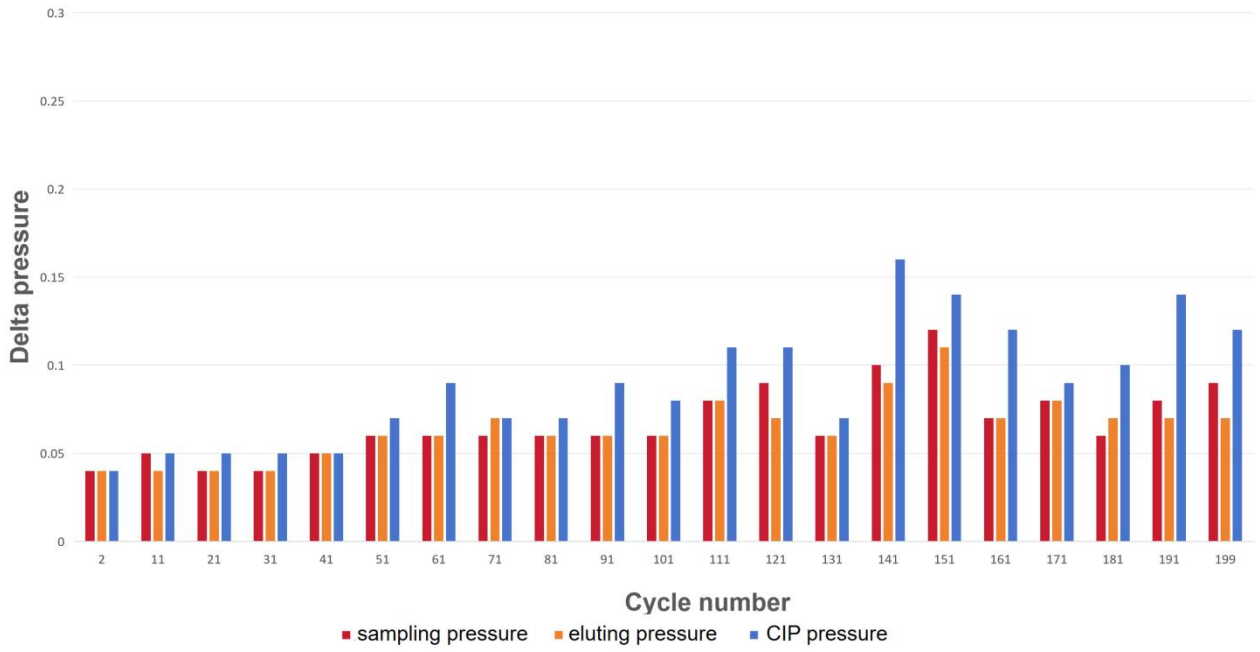


Fig. 5 TH-ProteinA Life Test Pressure Detection Data

2.6.6 HCP residue

The CHO cell antibody culture solution, which originally had a high HCP presence, was reduced to about 300 ppm after purification with TH-proteinA, and the HCP residue remained at a stable value after 200 cycles.

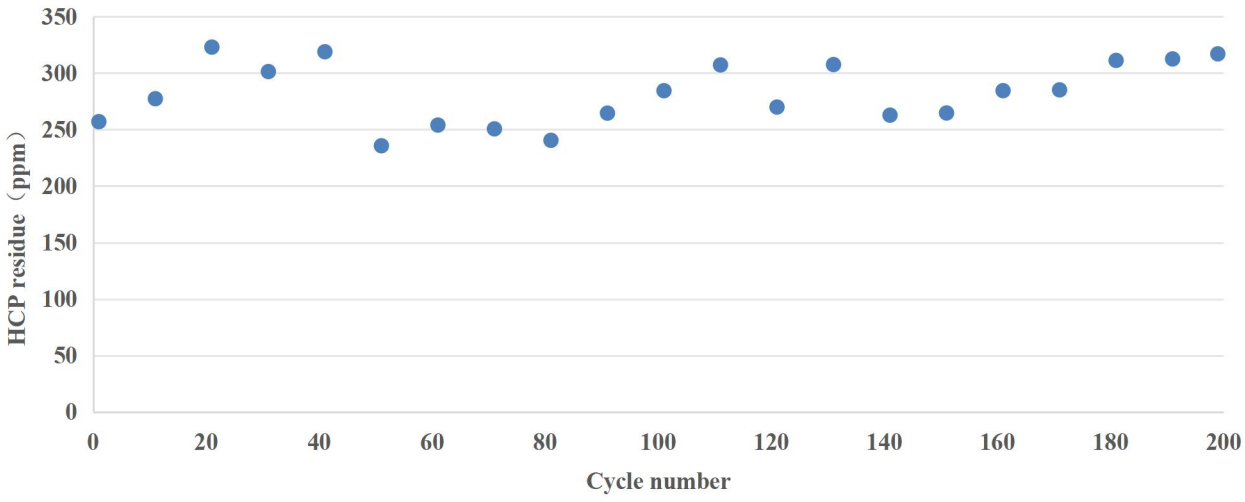


Figure 6 TH-ProteinA HCP residue assay data

2.6.7 Protein A ligand residue

After 200 cycles of TH-ProteinA use, the protein A ligand residue was within 10 ppm, and the protein A ligand residue showed a decreasing trend with the increase of the number of resin use.

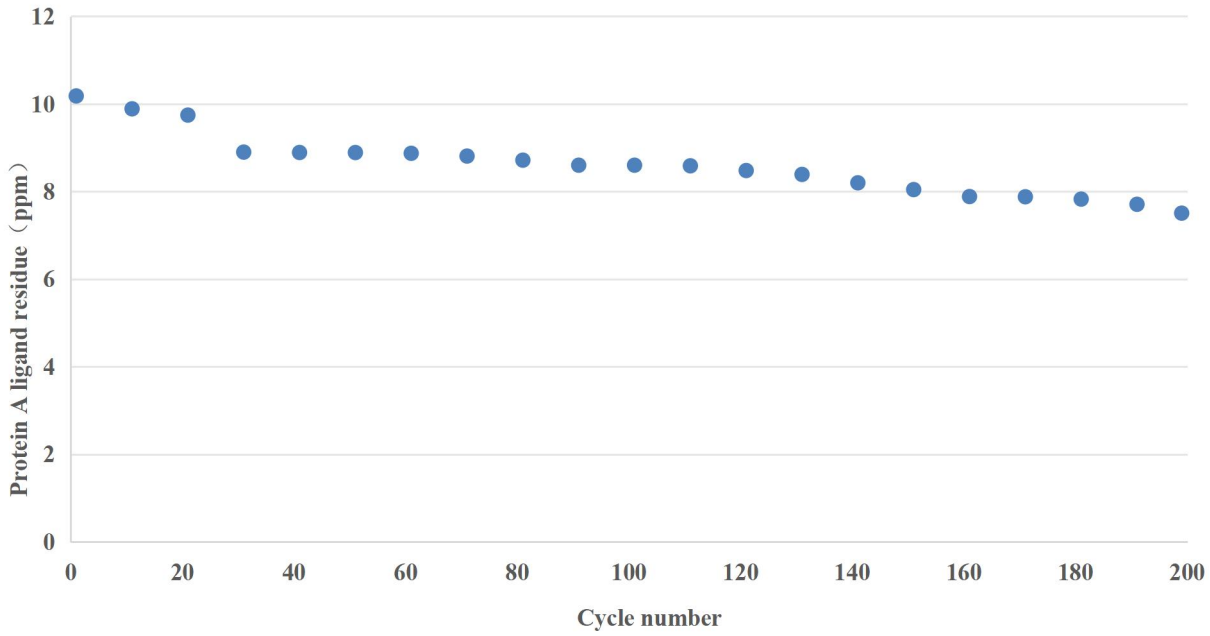


Figure 7 TH-ProteinA Protein A Ligand Residue Assay Data

2.7 Alkali resistance

TH-Protein A medium was statically placed in 0.1/0.5 M NaOH at room temperature, and the results are shown in Fig. 4, where the dynamic loadings were reduced by no more than 10% after 100 h of 0.1 M NaOH treatment; by no more than 20% after 24 h of 0.5 M NaOH treatment; and, by no more than 40% after 24 h of 1 M NaOH treatment.

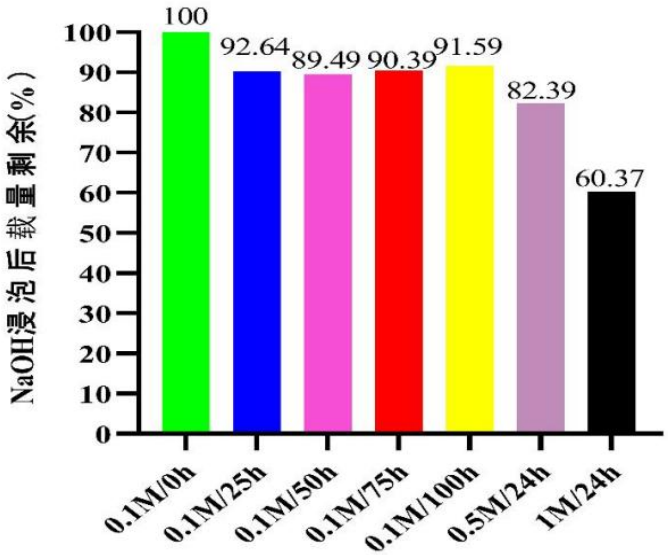


Fig. 8 Remaining load of TH-Protein A after soaking in sodium hydroxide for different times

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 can effectively prevent the accumulation of contaminants and maintain the stable working condition of the chromatography medium.Customers can determine the frequency of in-situ cleaning according to the degree of contamination of the media during use (if the contamination is more serious, it is recommended to carry out in-situ cleaning after each use to ensure the reproducibility of the results).

- The column can be washed for 2 column volumes with 2M NaCl to remove stronger non-specifically bound proteins;
- The column is then washed with 0.1-0.5M NaOH with a contact time of 10-15 min.
- Immediately rinse at least 5 column volumes with binding buffer.

4. Sterilization and storage

Since 20% ethanol or 2% benzyl alcohol preservation solutions are not bactericidal or pyrogenic, it is recommended that TH-Protein A media can be treated with 0.1M NaOH for 30min or 0.5M NaOH for 15min prior to and during use to reduce the risk of microbial contamination.

TH-Protein A is sold with 20% ethanol or 2% benzyl alcohol as preservation solution.After use, TH-Protein A should be stored in 20% ethanol at 2~8℃ in airtight condition.To prevent ethanol evaporation and microbial growth, it is recommended that the preservation solution be replaced with a fresh one every 3 months.

5. Destruction and recycling

Since TH-Protein A is difficult to degrade in nature, incineration is recommended for environmental protection.

6. Ordering Information

Table 4 Article number and packaging

Product	Item No.	Norm
TH-Protein A	Y5001	25mL
	Y5002	100mL
	Y5003	500mL
	Y5004	1L
	Y5005	5L
	Y5006	10L
	Y5007	20L