7th Edition, revised in April, 2017

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

MMA Focurose 6HP (MMA-6HP)

Catalog No: E-CM-IE20

This manual must be read attentively and completely before using this product.

May you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Please read this manual carefully before use to ensure the performance and successful operation. If you have any questions, please contact our Technical Support.

Product introduction

MMA-6HP is a kind of multi-mode biological separation medium, which is mainly used for moderate and fine purification of monoclonal antibody (to remove the Protein A* after Protein A purification, dimers, polymers, host cell proteins, nucleic acids), and it can also be used for fine purification of other biological molecules (to remove dimers, polymers, host cell proteins, nucleic acids, etc).

* Micro amount of Protein A may drop off in the process of operating Protein A-4HF.

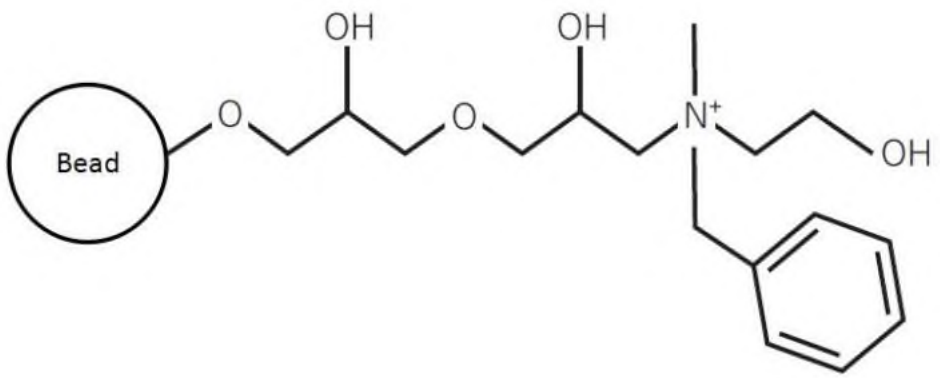


Figure 1: The ligand of MMA-6HP is a kind of multi-mode ligand, it has many types of interaction with target molecules, mainly include the ionic interaction (strong anion interaction), and followed by hydrogen binding and hydrophobic interaction, etc.

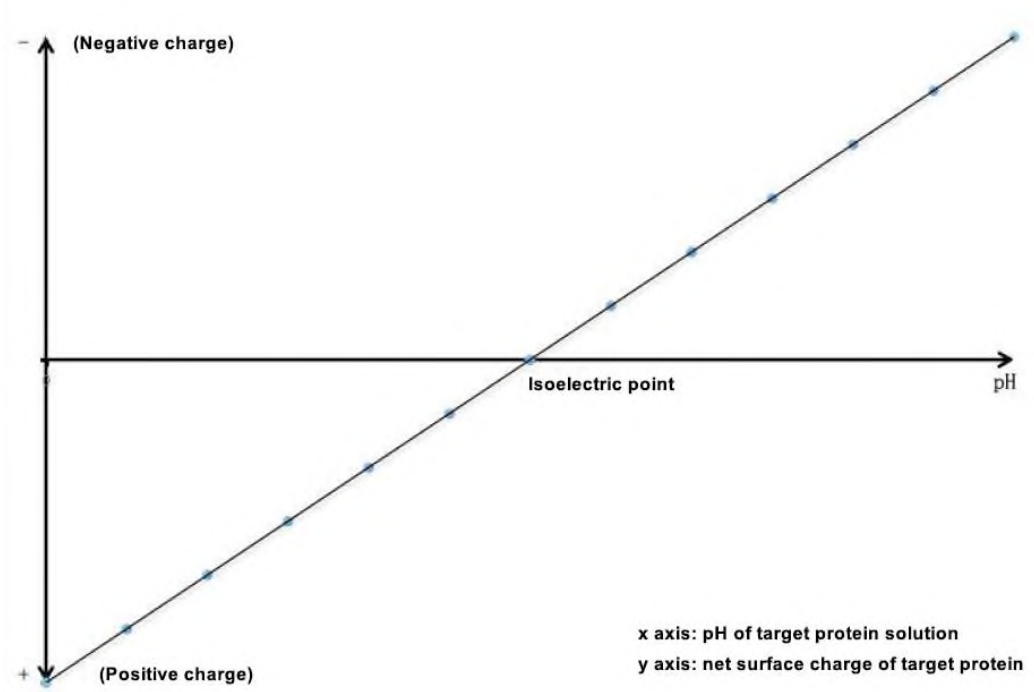
Advantages

1. Combine with charged molecules under high conductance conditions. The sample is no longer restricted by electrical conductivity and can processed ion exchange without pretreatment.
2. High binding capacity. It has a higher processing capacity under flowthrough mode.
3. It has a new selectivity compared with the traditional ion exchange media.
4. High resolution.

Table 1: Performance index

Matrix	Highly cross-linked 6% agarose
Particle size range	25-45 μm
Average particle size	37 μm
Media type	Strong anion exchanger
Ionic capacity	130-200 μmol Cl^-/mL (media)
Binding capacity	≥95mg BSA/mL (media)
pH stability	2-14 (short term) 3-12 (working) 3-12 (long term)
Chemical stability	All of the commonly used buffers, 1M acetate, 1M NaOH, 70% Ethanol, 8M urea, 6M guanidine hydrochloride. Avoid of oxidizing agent and anionic detergent
Flow rate	150-250 cm/h (0.3MPa, XK16/40. Column bed height:30 cm)
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30℃

Figure 2: Selection of ion exchange chromatographic media



Instruction:

1. Choose the cation exchange medium when pH of target solution < isoelectric point of target.
2. Choose the anion exchange medium when pH of target solution > isoelectric point of target.
3. When choosing an ion exchange medium, the pH of all the applied solutions should be within the application pH range of ion exchange media.
4. When choosing an ion exchange medium, the pH, salt type, and salt concentration of all the applied solutions should be able to maintain the activity of target, so as to avoid acid/ alkali hydrolysis and precipitation of target.

Selection of buffer

Table 2: Recommended buffers

pH range	Salt	Concentration (mM)
4.0-5.0	Acetate	20-100
4.0-6.0	Citrate	20-200
5.5-6.5	Bis-Tris	20-50
6.0-7.5	Phosphate	50-200
7.5-8.5	Tris	20-50
8.5-	Glycin-NaOH	20-100

Instruction:

1. The type and concentration of buffer solutions must be selected strictly according to Table 2.
2. Wrong buffer (type and concentration) may interfere the separation effect, which mainly reflects on the effects of separation degree, loading of ion exchange medium, pH fluctuation in separation and purification process.
3. When choosing an ion exchange medium, the pH of all the applied solutions should be within the application pH range of ion exchange media.
4. All buffer reagents must use reagents that are pure or of higher purity.
5. Solutions must be operated with filtration (particle size ≤ 45 μm, 0.22 μm. particle size ≤ 165 μm, 0.45 μm. particle size ≤ 300 μm, 0.8 μm. Avoid of blocking of ion exchange medium) and degassing (affect the separation effect).

Selection of purification mode

According to the purification mode, it can be classified into **adsorption mode** (target adsorption, impurity flowthrough) and **flowthrough mode** (target flowthrough, impurity adsorption).

Note: Flowthrough mode is recommended to selected preferential (higher processing capacity, e.g., moderate and fine purification of monoclonal antibody). Absorption mode is recommended to be selected under special conditions.

Preparation of sample

1. Adjust the pH (titration with low concentration of weak acid/ weak alkali or buffer exchange), detect the electric conductance in sample.
2. Filtration of sample (particle size ≤ 45 μm, 0.22 μm. particle size ≤ 165 μm, 0.45 μm. particle size ≤ 300 μm, 0.8 μm. Avoid of blocking of ion exchange medium).

Note: It is not recommended to directly adjust the pH of sample solution with strong acid/ strong alkali, which may lead to degradation and inactivation of target protein. Buffer exchange can be performed with gel filtration (G25), dialysis, and ultrafiltration.

Operation (MMA-6HP 1 mL or MMA-6HP 5 mL. Here is an operation of further fine purification of monoclonal antibody sample purified with Protein A-4HF for reference.)

1. **Sample preparation**

   Adjust the pH of sample to be 0.5 (Sample 1), 1.0 (Sample 2), 1.5 (Sample 3) and 2.0 (Sample 4) lower than mAb pI respectively, and record the conductance of samples.

   Note: The conductivity in the sample shall not exceed 25 mS/cm (dilute the samples if above this value), otherwise it will affect the adsorption of impurities and the medium.

2. **Preparation of equilibrium liquid**

   Select the appropriate buffer according to the Table 2 and prepare the equilibrium liquid. Adjust the pH and conductance to be consistent with the sample solution (by adding salt to improve the conductance). Prepare 4 types of equilibrium liquid in total.

   Note: Adjust the pH to be close to the final pH first, then slowly add salt to adjust the conductance, and finally adjust the pH and conductance finely.

3. **Preparation of eluent**

   0.05M NaAc, 1.0M NaCl, pH 5.0.

4. **Equilibration**

   Equilibrate the column with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (MMA-6HP 1 mL) or 2.0 mL/min (MMA-6HP 5 mL) until the baseline turns stable and then set to zero.

   Note: Equilibrate the columns with 4 corresponding equilibrium liquids.

5. **Sample application**

   Apply the sample with a flow rate of 0.2 mL/min (MMA-6HP 1 mL) or 1.0 mL/min (MMA-6HP 5 mL) (Sample 1). Collect the flowthrough solution when the absorbance begins to increase.

   Note: Apply the 4 sample solutions to the corresponding equilibrated columns.

6. **Wash**

   Wash out the impurities with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (MMA-6HP 1 mL) or 2.0 mL/min (MMA-6HP 5 mL) until the baseline returns to zero and stop collection.

   Note: Wash the applied columns with 4 corresponding equilibrium liquids.

7. **Elution**

   Elute with 5~10 CV of eluent with a flow rate of 0.5 mL/min (MMA-6HP 1 mL) or 2.0 mL/min (MMA-6HP 5 mL). Collect the flowthrough solution when the absorbance begins to increase. Stop collection when the baseline returns to zero.

8. Wash

Perform the washing and regeneration according to the operation of Step 7.

9. Analysis of results

Test the start material, folwthrough solution and eluted pool with SDS-PAGE and titer detection. Compare the results of 4 columns, the purification condition in the flowthrough solution with the highest recovery rate and purity is the best purification process.

Note: The purification condition (pH and conductance) can be adjusted according to the customer's requirements.

Cleaning

The excellent performance of media (e.g. loading ability, mobility, column efficiency, etc.) can be recovered after cleaning the strong coupling substance (e.g. some strong coupling protein, denatured protein, lipids, etc.).

It is recommended to wash the media after used for each 5 times. The exact washing frequency should be adjusted according to the cleanliness of the purified sample.

1. Wash the media with 20 CV of 0.1M NaAc (pH 3.0), then wash the media with 20 CV of purified water.

   Note: This procedure is used to remove the strong ionic binding substances.
2. Wash the media with 10 CV of 1M NaOH and let it stand for 0.5~1 hour, then wash the media with purified water to neutral.

   Note: This procedure is used to remove the precipitates, lipids and denatured substances accumulated in the media.
3. Store the media after washed with 5~10 CV of 20% ethanol.

   Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

Trouble shootings

Problem	Possible cause	Suggestion
The impurity does not combine with the media or the combining amount is low under flowthrough mode. The target compound does not combine with the media or the combining amount is low under adsorption mode	Overloading of sample volume.	Decrease the sample volume.
	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely and effectively.
	The impurities or target is uncharged or charged with the same with the media.	Select the appropriate binding buffer.
	Improper detergents were added to the sample.	Check if there is improper detergent in the sample.
	Poor binding condition.	Optimize the binding condition (pH and conductance).
No target compound was collected under adsorption mode	The target compound does not combine with the media or the combining amount is low.	Confirm whether the target combine with media or not.
	Unsuitable elution condition.	The elution capacity of the eluent is not enough. Adjust the pH or increase the salt concentration of eluent.
	Insufficient elution time.	Decrease the flow rate and prolong the retention time of eluent.
	Insufficient elution volume.	Increase the elution volume.
	The target compound accumulates in the elution buffer.	Check the stability of target compound in the elution buffer (salt concentration, pH, etc.)
Low purity of target compound	Sample has not been pretreated.	Samples must be centrifuged or filtered before loading.
	High viscosity of sample.	Dilute the sample properly with equilibrium liquid to decrease the viscosity.
	Insufficient washing under adsorption pattern.	Increase the washing volume until the baseline turns smooth and keep consistence with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.

	Elution conditions were poor under adsorption pattern.	Optimize the elution condition.
	The target compound degrades.	Determine the stability of target compound.
	Bad loading effect of column resin.	Reload or re-purchase.
	The top of separation column has a large volume of sample.	Reload the column or reduce the volume of the sample.
	There is microbial grow in the media.	Correctly store the media after used.
	The binding condition is poor under flowthrough pattern.	Optimize the purification process.
Decrease of loading volume	Speed of sample loading is too fast.	Reduce the flow speed of sample loading.
	Protein or lipids accumulate in the media.	Wash the media timely.
	Ligand was oxidized or dropped off due to excessive use.	Re-couple with new media
The chromatographic peak rises slowly	The media was loaded too tight.	Reload the column.
The chromatographic peak trails	The media was loaded too loose.	Reload the column.
The column bed cracks or being dry	Leakage occurred or a large volume of bubbles was introduced.	Check whether there is leakage or bubble, reload the column.
Flow of the column is exceedingly slow	Protein or lipids accumulate in the media.	Wash the media or filter membrane timely.
	Protein precipitates in the media.	Adjust the content of equilibrium liquid and wash buffer to maintain the stability of target compound and combining efficiency of media.
	There is microbial grow in the media.	Filter and degas all the reagents. Samples must be centrifuged or filtered before loading.