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TED Focurose 6FF

Catalog No: E-CM-AF26

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.

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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

TED Focurose 6FF can be used for separation and purification with the interactions between chelated metal ions and some amino acids (mainly include histidine, cysteine, tryptophan) on the side chain of protein, and it is suitable for separation and purification of His-tagged protein and biological molecules which interact with metal ions. The strong binding metal ions can be directly used for His-tagged protein expressed by eukaryotic expression system, and it have resistance for higher concentration of reductant and chelating agent. In addition, sample pretreatment is unnecessary. The cleaning and reviving of media are simple, which can be washed directly without nickel removal.

Advantages

1. Quick and easy (one-step purification).
2. Tolerate to higher concentration of reductant and chelating agent. His-tagged protein expressed by eukaryotic expression system can be loading without pretreatment, which can protect the protein activity to the utmost.
3. There is no need to remove nickel removal can wash with NaOH directly, which greatly shorten the cleaning period.
4. Multiple selection. Various metal ions (e.g. Cu^{2+}, Zn^{2+}, Fe^{2+}, Co^{2+}, Ca^{2+}, etc.) can be used.

Note: Avoid of using phosphate buffer when coupled with Ca^{2+} (precipitate will occur).

Performance index

Matrix	Highly cross-linked 6% agarose
Particle size range	45-165 μm
Average particle size	90 μm
Binding capacity	20 mg (His-tagged protein)/mL (media)
pH stability	3-12 (working) 2-14 (washing)
Chemical stability	0.01MHCl, 0.01M NaOH (1 week) 20 mM EDTA, 10 mM DTT, 1M NaOH, 8M Urea, 6M Guanidine hydrochloride (24 hours) 100 mM EDTA, 0.5M Iminazole (2 hours） 30% Isopropyl alcohol (20 minutes)
Flow rate	600 cm/h
Pressure	≤ 0.3 MPa
Storage buffer	20% Ethanol
Storage temperature	4~30℃（4~8℃ is preferred)

Chelation method

Different metal ions can be selected for chelation, and different metal ion solutions ($NiSO_4$/$CuSO_4$/$CoSO_4$/$ZnCl_2$/$CaCl_2$/$FeCl_2$) can be selected for different samples.

1. Wash with 5~10 CV (column volume) of purified water.
   Note: This operation is used to remove the 20% ethanol in media.
2. Wash with 5~10 CV of 0.1M metal ion solution, and then wash the media with 5~10 CV of purified water after standing for 0.5 hours.
   Note: This operation is used to couple and clean metal ions.
3. Wash with 5~10 CV of 20% ethanol and the store the media.
   Note: 20% ethanol can prevent the growth of microorganism. Media preserved with 20% ethanol can be stored at 4~30℃ (4~8℃ is preferred).

Operation (take 1 mL column and 5 mL column for example)

1. Wash (water)

Wash the media with 5~10 CV (column volume) of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This operation is used to remove the 20% ethanol in media.

2. Equilibration

Balance the media with 5~10 CV of equilibrium liquid with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) until the baseline turns stable and then set to zero.

Note: This procedure is used to balance the media. Make sure that the pH and component in media are in accordance with sample.

3. Sample application

Apply the sample with flow rate of 0.2 mL/min (1 mL) or 1.0 mL/min (5 mL) after centrifugation and filtration (0.45 μm). Wash with the equilibrium liquid until the baseline tends to zero.

Note: The binding capacity of proteins varies with the type of lysates, the properties of target proteins, flow rate, temperature, and pH. Low flow rates often increase the binding efficiency of samples.

4. Wash

Wash out the impurity substance with 5~10 CV of washing solution with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the washed solution.

Note: The washing solution is used to wash out some non-specific adsorbed impurity proteins.

5. Elution

Elute with 5~10 CV of eluent with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and collect the eluted solution.

Note: Low flow rate may increase the concentration of target protein in the eluted solution.

6. Wash with water

Wash the media with 5~10 CV of purified water with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL).

Note: This procedure is used to remove the eluent in media.

7. Storage

Wash the media with 5~10 CV of 20% ethanol with a flow rate of 0.5 mL/min (1 mL) or 2.0 mL/min (5 mL) and the store the media.

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

8. Preparation of buffer

Add 8M urea or 6M guanidine hydrochloride to the following equilibrium liquid, washing buffer and eluent if the sample is inclusion body.

Equilibrium liquid: 0.02M PB, 0.5M NaCl, adjust the pH to 7.4. Store the prepared equilibrium liquid at room temperature.

Note: NaCl in equilibrium liquid is used to inhibit the ion exchange of media.

Washing buffer: 0.02M PB, 0.5M NaCl, 0.005-0.01M iminazole, adjust the pH to 7.4. Store the prepared washing buffer at room temperature.

Note: Add 0.005-0.01M Iminazole to the washing buffer (yield preferred) or to the equilibrium liquid directly (purity preferred) according to the final application demands. It is not recommended to add 0.005-0.01M Iminazole into the equilibrium liquid when purifying denatured samples, or the binging level and loading amount will decrease.

Eluent: 0.02M PB, 0.5M NaCl, 0.5M Iminazole, adjust the pH to 7.4. Store the prepared eluent at room temperature.

Note: 0.02-0.10M Iminazole in eluent is enough to elute the target protein in general condition.

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 5~10 CV of purified water.

   Note: This procedure is used to remove the eluent (wash after using) or 20% ethanol (wash before using).

2. Wash the media with 5~10 CV of 1M NaOH and stand for 0.5~1 hour, then wash the media until the pH to neutral with 10~20 CV of purified water.

   Note: This procedure is used to remove the precipitated proteins, hydrophobic binding proteins, lipids or other impurity materials accumulated in 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 target compound does not combine with the media or the combining amount is low when purifying	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 expression condition is too severe that the His-tag was enwrapped and cannot combine with the media.	An empty vector is suggested to set as control of expression and purification to check whether the expression condition is suitable.
	The initial sample does not contain His-tagged protein.	Verify through the gene sequence or His-tag antibody.
	The target protein occurs in the flowthrough.	The target protein has not been expressed successfully, or the pH and components in sample and equilibrium liquid were incorrect
No target compound was collected or only a small amount of target compound was collected	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.	Increase the iminazole concentration in eluent.
	Insufficient elution time.	Decrease the flow rate and prolong the retention time of eluent.
	Insufficient elution volume.	Increase the elution volume.
	Weak elute ability of elution.	Increase the iminazole concentration in eluent.
	Target protein was washed out during the washing.	Decrease the iminazole concentration in washing buffer.
	The target compound accumulates in the elution.	Check the stability of target compound in the elution (salt concentration, pH, etc.) Add some additives into the eluent, such as 0.2%Triton X-100 or 0.5% Tween 20.
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.	Increase the washing volume until the baseline smooth and keep consistence with equilibrium liquid.
	Impurity protein or lipids accumulate in the media.	Wash the media timely and effective.
	Impurity substances have strong-affinity with Ni^{2+}.	Purify with other type of media (e.g. ion–sieve or molocular-sieve).
	The target compound degrades.	Determine the stability of target compound and add protease inhibitors.
	Bad loading effect of column resin.	Reload or re-purchase.
	Non-specificity absorption of impurity materials.	Add appropriate additive to reduce the non-specific absorption, such as 0.5%Triton X-100, 1.0% Tween 20 or 50% glycerol.
	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.
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 dropped off due to excessive use.	Re-couple with new media.
	The expression condition is too severe that the His-tag was enwrapped and cannot combine with the media.	An empty vector is suggested to set as control of expression and purification to check whether the expression condition is suitable.
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.