HisSep-250™ Spin Column
Expanded Capacity
Instructions

Description:

HisSep-250 spin columns contain a proprietary resin that provides a rapid, efficient, and totally non-denaturing method for the removal of histidine from formulation buffers of monoclonal antibodies and antibody drug conjugates (ADC). The high sample capacity of these columns can be 100 – 150 µL with up to 95% recovery. Each column contains a special low non-specific adsorbance frit with pre-packaged dry resin and requires 2 included collection tubes.

HisSep-250 spin columns have expanded capacity and are optimized specifically for the removal of histidine from biologics formulation buffers. The table below shows typical recoveries of mAbs with various sample volumes.

Sample Volume (µL)	Recovery (%)
100	86
125	90
150	95

Instructions:

A: Additional equipment and material needed

1. A fixed-angle-rotor microcentrifuge with adjustable speed and g-force.
2. Rehydration buffer: see the following step for the instructions for preparation.

B: Rehydration Buffer (20 mM Phosphate Buffer, pH 6.0) Preparation

1. Make a stock solution A: 0.2 M monobasic sodium phosphate, monohydrate (27.6g/L).
2. Make a stock solution B: 0.2 M dibasic sodium phosphate, anhydrous (28.4 g/L).
3. Mix 87.7 mL of A and 12.3 mL of B and dilute to a total volume of 200 ml, to make a 0.1 M, pH 6.0 phosphate buffer.
4. Dilute 20 mL of 0.1M phosphate buffer to 100 mL with DI water to make the rehydration buffer: 20 mM phosphate buffer, pH 6.0. The rehydration buffer is stable for one month at room temperature.

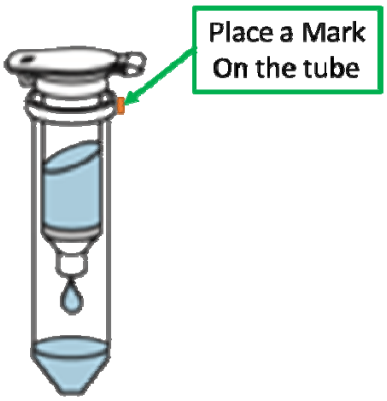
C: Rehydration HisSep250 Column (*Please note that rehydration of HisSep250 differs from HisSep)*

1. Gently tap the column or spin at 800 x g for 1-2 seconds to insure settling of the dry resin at the bottom of the spin column.
2. Place the spin column in a collection tube. Remove the cap from the column and add 700 µL of rehydration buffer. Replace the column cap and vortex the column with the collection tube vigorously for 15 seconds.
3. Remove the spin column from the collection tube. Invert the tube causing the partially wetted resin and liquid to fall completely to the cap portion of the tube.
4. Invert the tube again, causing the wetted resin and liquid to fall completely to the bottom portion of the tube.
5. Place the spin column into the collection tube.
6. Remove air bubbles by tapping the column and the collection tube together.

7. Allow column to rehydrate for at least 45 minutes at room temperature.

Note: It is important to fully hydrate the dry resin before use. Rehydrated columns can be stored at 4^0C for several days. Allow refrigerated columns to warm to room temperature before use.

8. After the resin has been completely hydrated, tap the bottom of the column with the collection tube together to remove the air bubbles if needed.
9. Open the cap and spin the column with the collection tube for 2 minutes at 800 x g to remove the excess rehydration buffer. If there is a drop remaining at the end of the column, blot it dry. Discard the liquid from the collection tube.
10. Place a mark on the side of the column where the compacted resin is slanted upward. In all subsequent centrifugation steps, place column in centrifuge with the mark facing outward. The highest point of the gel media in the column should always point toward the outside of the rotor.



Note: After step 6, do not keep the rehydrated column for a long time. Columns do not operate efficiently if they have dried out.

D: Sample loading and collection

1. Place column in a new collection tube, carefully apply 100-150 µl of sample to the center of the rehydrated resin bed without disturbing the gel surface.

2. Centrifuge the column with the collection tube at 800 × g for 2 minutes to collect the sample. Discard the column after use. Now the sample is ready for downstream analysis.