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Product Information

Seppro® Rubisco Spin Columns

Catalog Number **SEP070**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
The Seppro® Rubisco Spin Columns are based on avian antibody (IgY)-antigen interactions and optimized buffers for sample loading, washing, eluting, and column regeneration. They are specifically designed to remove D-Ribulose 1,5-Diphosphate Carboxylase (Rubisco) from plant samples. Rubisco catalyzes the first major step in carbon fixation and is the most abundant protein found in plants. It accounts for ~40% of the protein in green leaves and is a major obstacle in plant proteomics.

Rubisco is removed by the immobilized specific IgY when filtered biological samples are passed through the column. Selective immunodepletion of the highly abundant rubisco provides enriched flow-through fractions of low abundance proteins for further study and downstream proteomics analysis. The removal of Rubisco enables improved resolution and dynamic range for one-dimensional gel electrophoresis (1DGE), two-dimensional gel electrophoresis (2DGE), and liquid chromatography/mass spectrometry (LC/MS). The collected flow-through fractions may need to be concentrated dependent upon the downstream applications.

Characteristics of Rubisco Spin Columns
The column resin can be used 100 times. However, the columns may get clogged due to the insoluble materials from the samples. It is recommended to transfer the resin to a fresh spin column every 25 uses.

Capacity: ~0.2 mg of Rubisco
(Total protein mass removal)

Operating temperature: 18–25 °C

Shipping Buffer: 1× Dilution Buffer with 0.02% sodium azide

Components

Seppro Rubisco Spin Columns (Catalog Number S5449)	2 each
10× Dilution Buffer Tris-Buffered Saline (TBS) - 100 mM Tris-HCl with 1.5 M NaCl, pH 7.4 (Catalog Number S4199)	1 × 200 ml
10× Stripping Buffer 1 M Glycine, pH 2.5 (Catalog Number S4324)	1 × 200 ml
10× Neutralization Buffer 1 M Tris-HCl, pH 8.0 (Catalog Number S4449)	1 × 80 ml
2 ml Collection Tubes (Catalog Number T5449)	2 × 500 each
Empty Spin Columns (Catalog Number S4574)	6 each

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Preparation of 1× concentration buffers - Separately dilute the three 10× buffers (Dilution, Stripping, and Neutralization Buffers) 10-fold with water. If precipitation occurs in the 10× buffers, allow the bottle to warm to room temperature and mix until completely dissolved prior to use. **Do not dilute all of the 10× Neutralization Buffer,** save a volume of the 10× neutralization buffer for neutralization of eluted bound proteins if analysis of bound proteins is desired.

Sample Preparation– It is not recommended to load an unfiltered plant protein extract directly onto the column. Dilute the plant extract in 1× Dilution Buffer to a final volume between 500–650 µl. (If the volume of a diluted sample is more than 650 µl, transfer half of the prepacked beads into a new empty spin column and load 50% of suggested capacity each for the two columns).

It is suggested to **avoid using reducing reagents, such as DTT, BME, or denaturing reagents, such as urea or guanidine-HCl in the sample extracts**. Remove particulates with a 0.45 µm spin filter, centrifuge for 1 minute at 10,000 × *g*.

Storage/Stability

Store the columns at 2–8 °C. After use, equilibrate the columns with 1× Dilution Buffer containing 0.02% sodium azide and store the columns at 2–8 °C with the end-caps tightly sealed. **Do Not Freeze** the columns.

Procedure

Note: Before using the columns for samples, it is suggested to run one or two blank samples (buffer only) to remove any residual non-covalently bound IgY from the beads.

Immunocapture of Rubisco

1. Snap off the tip from the spin column and place the column in a 2 ml Collection Tube.
2. Centrifuge the column for 30 seconds at 2,000 rpm in a microcentrifuge onto obtain dried beads.
3. Place the end cap on the column tip. Immediately add 0.5 ml of diluted sample on the dried beads in the column. Seal the column with the top snap cap. Note: The sample loading may need to be adjusted. If the depletion is not complete, less sample loading is recommended.
4. Mix the beads and the sample completely by inversion and shaking the column, place it on an end-to-end rotator, and incubate at room temperature for 15 minutes.
5. Invert the column. Remove the end cap, place the column in a 2 ml Collection Tube, and centrifuge for 30 seconds at 2,000 rpm. Collect flow-through sample for further analysis.
6. To obtain maximum yield of the flow-through sample, an optional wash step can be applied. Add 0.5 ml of 1× Dilution Buffer to the beads. Mix beads and buffer completely by inversion and shaking the column. Centrifuge for 30 seconds at 2,000 rpm. Collect and combine with the flow-through sample from step 5 for further analysis.

Elution of Bound Protein(s)

1. To remove proteins non-specifically bound to beads, wash beads with 1× Dilution Buffer, a total of 3 times. For each wash, always first insert the end cap, then add 0.5 ml of 1× Dilution Buffer, and seal the column with top snap cap. Mix the beads and buffer completely by inversion and shaking the column, remove the end cap while inverting the column, and place it in a 2 ml Collection Tube. Centrifuge for 30 seconds at 2,000 rpm and save the flow-through for further analysis.
2. Strip off bound proteins from beads using 1× Stripping Buffer, a total of 4 times within 15 minutes. For each stripping, place the end cap on the column first after centrifugation, then add 0.5 ml of 1× Stripping Buffer, and seal the column with the top snap cap. Mix the beads and buffer completely by inversion and shaking the column, incubate at room temperature for 3 minutes, remove the end cap while holding the column upside down, and place it in a 2 ml Collection Tube. Centrifuge for 30 seconds at 2,000 rpm and collect the eluate. It is **crucial for column stability to immediately neutralize the beads** (see Regeneration of Column Resin).
3. Pool four eluted samples (~2 ml) and neutralize with 200 µl of 10× Neutralization Buffer. Samples can be further concentrated using a centrifugal filter device to desired concentration and volume.

Regeneration of Column Resin

1. To regenerate the spin column after stripping bound proteins, **immediately** neutralize the beads with 0.6 ml of 1× Neutralization Buffer. Mix beads and buffer completely by inversion and shaking the column. Incubate at room temperature for 5 minutes.
2. Spin down beads in the column for 30 seconds at 2,000 rpm.
3. Resuspend used beads in 0.5 ml of 1x Dilution Buffer. Beads are ready for storage at 2–8 °C or next separation. For storage of regenerated beads, it is suggested that the storage buffer contain 0.02% sodium azide.
   Notes: The column resin can be recycled 100 times. However, the column may get clogged due to the insoluble materials from the samples. It is recommended to transfer the resin to a fresh spin column every 25 uses.

   If the column capacity decreases, apply a buffer only cleaning run to remove residual proteins bound to the column.

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