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Product Information

Seppro® Rubisco Tips

Catalog Number **S3949**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Seppro® Rubisco Tips are based on avian antibody (IgY)-antigen interactions and optimized buffers for sample loading, washing, eluting, and tip regeneration (available separately). The tips 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 crude biological samples are passed through the tip. 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 Tips

The tips are designed for use with the automated Magtration® System SA-1 from Precision System Science USA. Twelve tips are simultaneously operated and twelve samples can be processed at once with no hand-on manipulations in ~65 minutes.

Tip Size: 0.5 ml bed volume

Tip capacity: ~0.4 mg of Rubisco

Operating temperature: 18–25 °C

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

Shipping temperature: 2–8 °C

Tip body materials: Polycarbonate tip and polyethylene frits

Usage: Tips may be used 30 times.

Component

Seppro Rubisco Tips (Catalog Number S3949)	6 each

Reagents and Equipment Required for the Depletion Process, but Not Provided

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× Neutralization Buffer 1 M Tris-HCl, pH 8.0 (Catalog Number S4449)	80 ml
Pre-filled Reagent Cartridges (Catalog Number S6574)	48 each
Corning® Spin-X® Centrifuge tube filters 0.45 µm, pack of 100 (Catalog Number CLS8163)	1 × pack

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.

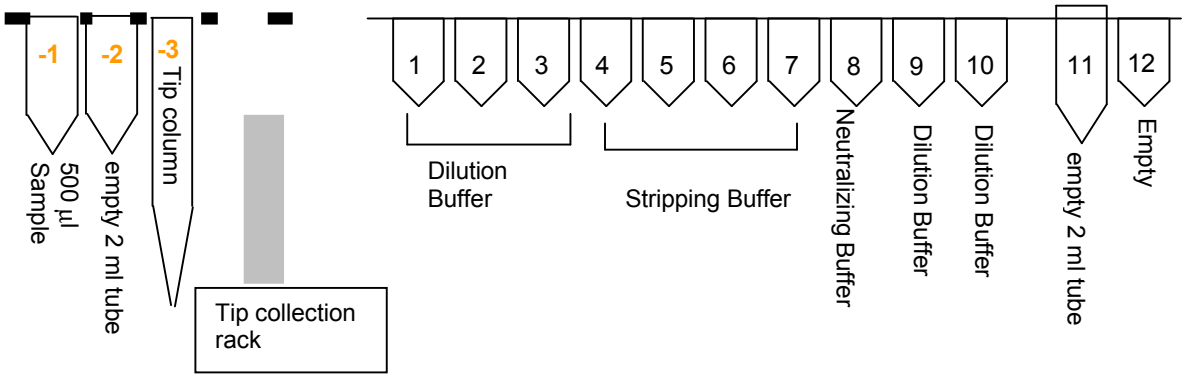
Storage/Stability

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

Preparation Instructions
Preparation of 1× Dilution Buffer - Dilute 10× Dilution Buffer 10-fold with water. Use 1× Dilution Buffer for sample preparation.

Use 10× Neutralization Buffer to neutralize 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 tip. Dilute the sample using 1× Dilution Buffer to a final volume of 500 µl. Samples may contain particulate materials, which can be removed by centrifugation at 10,000 rpm for 1 minute **or** with the use of a 0.45 µm spin filter with centrifugation for 1 minute at 9,000 × *g*. 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.



Sample/Tip Rack ***Pre-filled/open reagent cartridge***

The Buffer Arrangement in Pre-filled Cartridges and Tubes:

Position –1:	0.5 ml of unfractionated sample in 2 ml screw cap tube
Position –2:	Empty 2 ml screw cap tube
Position –3:	tip with tip holder
Wells 1–3:	1.0 ml of Dilution Buffer (Pre-filled Cartridge)
Wells 4–7:	1.0 ml of Stripping Buffer (Pre-filled Cartridge)
Well 8:	1.0 ml of Neutralizing Buffer (Pre-filled Cartridge)
Wells 9–10:	1.0 ml of Dilution Buffer (Pre-filled Cartridge)
Position 11:	Empty 2 ml screw cap tubes
Position 12:	Empty. Used for buffer retention.

Note: Buffers in wells are Pre-filled Cartridges.

Procedure
Notes: Do not expose tips to organic solvents (like alcohols, acetonitrile, etc.), strong oxidizers, acids, or reducing agents and other protein denaturing agents.

Refer to Operation Manual for set-up of Magtration System SA-1 instrument, accessories, and disposables.

Pretreatment of Tips – "mock run" to remove any residual non-covalently bound IgY from the beads.

1. Turn on SA-1and lift the door for reagent loading.
2. Load the Reagent Cartridges into the cartridge rack according to the number of the samples and tips to be used (SA-1 holds a maximum of 12 tips for 12 samples/per run).
3. Place the cartridge rack into the rack setting position on the stage.
4. Remove the top cap of the tip, leave the end cap with it, and put it at position –3 without tip sheath.
5. Set tip/tube rack into the instrument.
6. Insert the Tip Collection Rack at position –4

7. Close the door and follow the instructions on the display screen of the instrument to start the Mock Run (select 2) procedure for the process, see Table 1. It will take ~30 minutes to finish the process.
   Note: After each run, remove both racks from the stage, replace all used cartridges and tubes. Take out tips with end caps on from tip collection rack and put them at position –3. Set racks back on the stage.

Table 1.
Mock Run
<Tip/tube rack>

Position –3	Seppro Tip in tip sheath
Position –2	Empty
Position –1	Empty

<Cartridge Rack>

Well 1	Equilibrium 1
Well 2	Equilibrium 2
Well 3	Equilibrium 3
Well 4	Elution 1
Well 5	Elution 2
Well 6	Elution 3
Well 7	Elution 4
Well 8	Neutralization
Well 9	Equilibrium 4
Well 10	Equilibrium 5
Position 11	Empty
Well 12	Retention liquids

Sample Depletion

1. Put the tube containing 500 µl of the plant extract sample in a 2.0 screw cap tube at position –1. Put an empty 2 ml tube at position –2. Put a 2 ml tube at position 11.
2. Start the depletion procedure (select 1) for sample processing (see Table 2).
3. After completing the procedure, neutralize the eluted bound fractions by adding 100 µl of 10× Neutralization Buffer into the tube at position 11 (Elutions 1 & 2), well 4 (Elutions 3 & 4), and well 5 (Elutions 5 & 6); and 50 µl into well 6 (Elution 7) and well 7 (Elution 8).
4. Transfer desired fractions (such as neutralized Elutions 3–8) to collection tubes. Store properly for downstream analysis.
5. Store the tip with 1× Dilution Buffer at 2–8 °C. For long-term storage, use 1× Dilution Buffer with 0.02% sodium azide. **Do Not Freeze** the tips.

Table 2.
Depletion Run
<Tip/tube rack>

Position –3	Seppro Tip with end caps on
Position –2	Empty 2 ml tube. (After the process, this well contains ~0.5 ml of Wash 1).
Position –1	Sample 500 µl in 2 ml tube

<Cartridge Rack>

Well 1	Wash 2	
Well 2	Wash 3	
Well 3	Wash 4	
Well 4	Elution 1	**(after the process, this well contains Elutions 3 and 4)**
Well 5	Elution 2	**(after the process, this well contains Elutions 5 and 6)**
Well 6	Elution 3	**(after the process, this well contains Elution 7)**
Well 7	Elution 4	**(after the process, this well contains Elution 8)**
Well 8	Neutralization	
Well 9	Equilibrium 1, 2	
Well 10	Equilibrium 3	
Position 11	2 ml tube	**(after the process, this well contains Elutions 1 and 2)**

Well 12 Retention liquids

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