INSTRUCTIONS

HiPPR™ Detergent Removal Resin

88305 88306 88307 2300.3

Number	Description
88305	**HiPPR Detergent Removal Spin Column Kit,** 5mL of resin supplied as 50% slurry (e.g., 5mL of settled resin is equivalent to 10mL of 50% slurry) in 0.15M sodium chloride and 0.05% sodium azide, with two Spin Column Accessory Packs, 54 columns
88306	**HiPPR Detergent Removal Spin Columns,** 24 columns, each column contains ~100µL of resin supplied as 25% slurry (e.g., 100µL of settled resin is equivalent to 400µL of 25% slurry) in 0.15M sodium chloride and 0.05% sodium azide
88307	**HiPPR Detergent Removal 96-Well Filter Plate Kit,** each well contains ~100µL of resin supplied as 50% slurry (e.g., 100µL of settled resin is equivalent to 400µL of 25% slurry) in 0.15M sodium chloride and 0.05% sodium azide

Storage: Upon receipt store at 4°C. Products shipped at ambient temperature.

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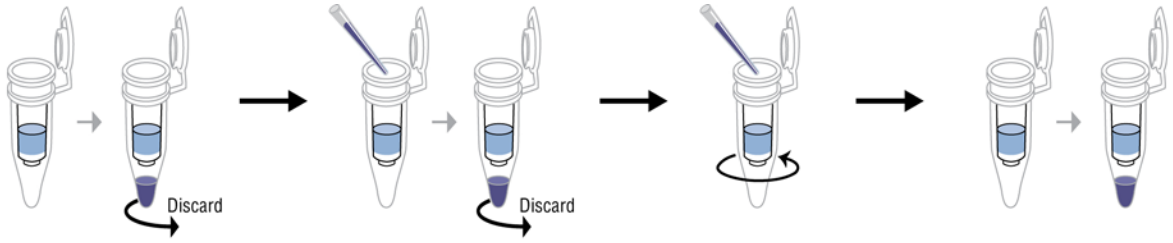
Introduction

The Thermo Scientific HiPPR (High Protein and Peptide Recovery) Detergent Removal Resin removes commonly used ionic, nonionic and zwitterionic detergents from samples with low protein/peptide concentrations (1 to 100µg/mL). Detergents and surfactants are used for solubilizing, stabilizing and disaggregating proteins and protein complexes; however, detergents interfere with downstream analyses such as ELISA, isoelectric focusing and mass spectrometry (MS). The HiPPR Detergent Removal Resin removes detergents with > 95% efficiency at concentrations of 0.5-1%, enabling recovery and characterization of low-abundant proteins. The columns and 96-well filter plates are pre-loaded with detergent-removal resin for multiple-sample processing of 25 to 100µL.

Procedure Summary



1. Add detergent removal resin (25-200µL) into Micro Spin Column and centrifuge for 1 minute at 1500 x *g* to remove the storage buffer. Discard the flow-through.
2. Add 25-200µL equilibration buffer, centrifuge at 1500 x *g* for 1 minute and discard the flow-through. Repeat 2 additional times.
3. Add detergent-containing sample (25-200µL), mix and incubate for 10 minutes at RT.
4. Centrifuge at 1500 x *g* for 2 minutes to collect the detergent-free sample for downstream applications.

Important Product Information

- The sample must be added directly onto the resin to remove detergents with efficient protein/peptide recovery.
- To remove the column's bottom plug, pull out the plug in one continuous motion without twisting.
- Use one part sample to one part settled resin (e.g., 50µL of sample to 50µL of settled resin) for removing SDS, sodium deoxycholate, Triton™ X-100, Triton X-114 and NP-40. Please refer to Table 1 and 2 for the maximum removable detergent concentrations in the spin-column and plate formats, respectively.
- Use one part sample to two parts settled resin (e.g., 50µL of sample to 100µL of settled resin) for removing CHAPS, octyl glucoside and octyl thioglucoside. Please refer to Table 1 and 2 for the maximum removable detergent concentrations in the spin-column and plate formats, respectively.

Table 1. Maximum removable detergent concentrations using the Thermo Scientific HiPPR Detergent Removal Spin Column.

Detergent	Maximum Removable Concentration (%)
SDS	1
Sodium deoxycholate	1
Triton X-100	1
Triton X-114	0.5
NP-40	0.75
CHAPS*	1
Octyl glucoside*	1
Octyl thioglucoside*	1

*Use one part sample to two parts settled resin

Table 2. Maximum removable detergent concentrations using the Thermo Scientific HiPPR Detergent Removal 96-well Filter Plate.

Detergent	Maximum Removable Concentration (%)
SDS	0.4
Sodium deoxycholate	0.5
Triton X-100	0.25
NP-40	0.125
CHAPS*	1
Octyl glucoside*	1
Octyl thioglucoside*	1

*Maximum processable sample volume is 50µL

Detergent Removal Procedure using Spin Columns

Materials Required

- Variable-speed bench-top microcentrifuge with a minimum speed of 1500 × *g*
- 2mL microcentrifuge collection tubes, multi-channel pipettor and tips
- Equilibration buffer: use a buffer such as AMBIC, PBS, MES, MOPS, Tris or carbonate-bicarbonate at pH 5 to 10. Do not use buffers containing organic solvents.

A. Method

1. Place an empty spin column into a 2mL collection tube. If using a pre-dispensed HiPPR Detergent Removal Column (Product No. 88306), remove the bottom plug, place the spin column into a 2mL collection tube and proceed to step 5.
2. Gently swirl the bottle of HiPPR Detergent Removal Resin to obtain an even suspension.
3. Using a cut pipette tip, add an appropriate amount of resin slurry into the spin column (See Important Product Information).
4. Centrifuge at 1500 × *g* for 1 minute to remove storage buffer. Discard the flow-through.
5. Add the appropriate volume of equilibration buffer (one part buffer to one part settled resin), centrifuge at 1500 × *g* for 1 minute and discard the flow-through. Repeat this step two additional times.
6. Insert bottom plug and place column into a new collection tube.
7. Slowly apply sample directly onto the top of the compacted resin bed. Cap the column, vortex gently to mix the sample with the resin and incubate at room temperature for 10 minutes.
8. Slightly loosen the cap, remove the bottom plug and place the column in a collection tube. Centrifuge at 1500 × *g* for 2 minutes to collect the detergent-free sample. Discard the used column.

Detergent Removal Procedure using a 96-well Filter Plate

Materials Required

- Centrifuge with a minimum speed of 1000 × *g* and rotor and carriers capable of handling staked plates with a height of 4.4cm.
- Equilibration buffer: use a buffer such as AMBIC, PBS, MES, MOPS, Tris or carbonate-bicarbonate at pH 5 to 10. Do not use buffers containing organic solvents.

A. Method

1. Equilibrate the HiPPR Detergent Removal 96-well Filter Plate to room temperature.
2. Carefully remove the sealing material from the bottom of the plate. Place the detergent removal plate on top of a wash plate.
3. Remove the sealing material from the top of the plate.
4. Place the assembly into a centrifuge with a 96-well plate-carrier rotor and centrifuge at 1000 × *g* for 2 minutes to remove the storage buffer. Discard the flow-through and replace the detergent-removal plate on top of the wash plate.
5. Add 200µL of equilibration buffer to each well and centrifuge at 1000 × *g* for 2 minutes. Discard the flow-through. Repeat this step two additional times.
6. Stack the detergent-removal plate on top of a sample-collection plate (blue), aligning the alphanumeric indices on the plate.
7. Slowly apply sample (25-100µL) to the center of the resin bed. To expel the entire sample from the pipette tip, carefully touch the top of the resin. Cover the plate with a new plate sealer and incubate for 10 minutes at room temperature.

   Note: To process > 96 samples, evenly divide the samples between two plates. To process ≤ 96 samples, balance the centrifuge by using an unprocessed plate with the top and bottom seal in place.
8. Centrifuge the plate assembly at 1000 × *g* for 2 minutes to collect the detergent-free sample. Discard the detergent removal plate or reserve it for future balancing purposes.

Troubleshooting

Problem	Possible Cause	Solution
Sample or buffer does not flow through the resin or plate	Centrifugation problem	Ensure the centrifuge is working properly
	Bottom closure was not removed	Ensure bottom closure is removed
Detergent is not completely removed	Improper sample loading	Load sample directly onto the top of the resin bed
	Insufficient amount of resin used	Use the appropriate resin amount (See Important Product Information) or slightly increase the resin amount (e.g., increase from 1 part sample:2 parts settled resin to 1 part sample:1.5 parts settled resin)
	Exceeded the maximum removable detergent concentration	Ensure the detergent concentration is appropriate or dilute sample before processing (See Table 1 or 2)
	Buffer contained organic solvents	Remove organic solvents by dialysis before detergent removal

Additional Information

A. Typical detergent removal efficiency and protein recovery using Thermo Scientific HiPPR Detergent Removal Resin.†

Detergent	Sample Volume (µL)	Detergent Removal (%)	Protein Recovery (%)
SDS (1%)	25	> 99	98
	50	> 99	97
	100	> 99	100
	200	> 99	100
Triton X-100 (1%)	25	98	82
	50	95	86
	100	98	86
	200	98	93
NP-40 (0.75%)	25	95	90
	50	96	94
	100	97	91
	200	97	97
CHAPS (1%)	25	95	64
	50	97	70
	100	98	78
	200	98	75

† A volume of 0.1mL of BSA (1.5µg) with detergent in 0.15M sodium chloride and 0.05% sodium azide was mixed with 0.1mL of detergent removal resin (0.2mL for CHAPS removal). Residual SDS was measured using Stains-All dye.[1] Triton X-100 and NP-40 were measured by absorbance at 275nm (protein absorbance was subtracted); CHAPS, sodium deoxycholate, octyl glucoside and octyl thioglucoside were measured by colorimetric method using concentrated sulfuric acid and phenol.[2] The Thermo Scientific Micro BCA Protein Assay was used for protein determination.

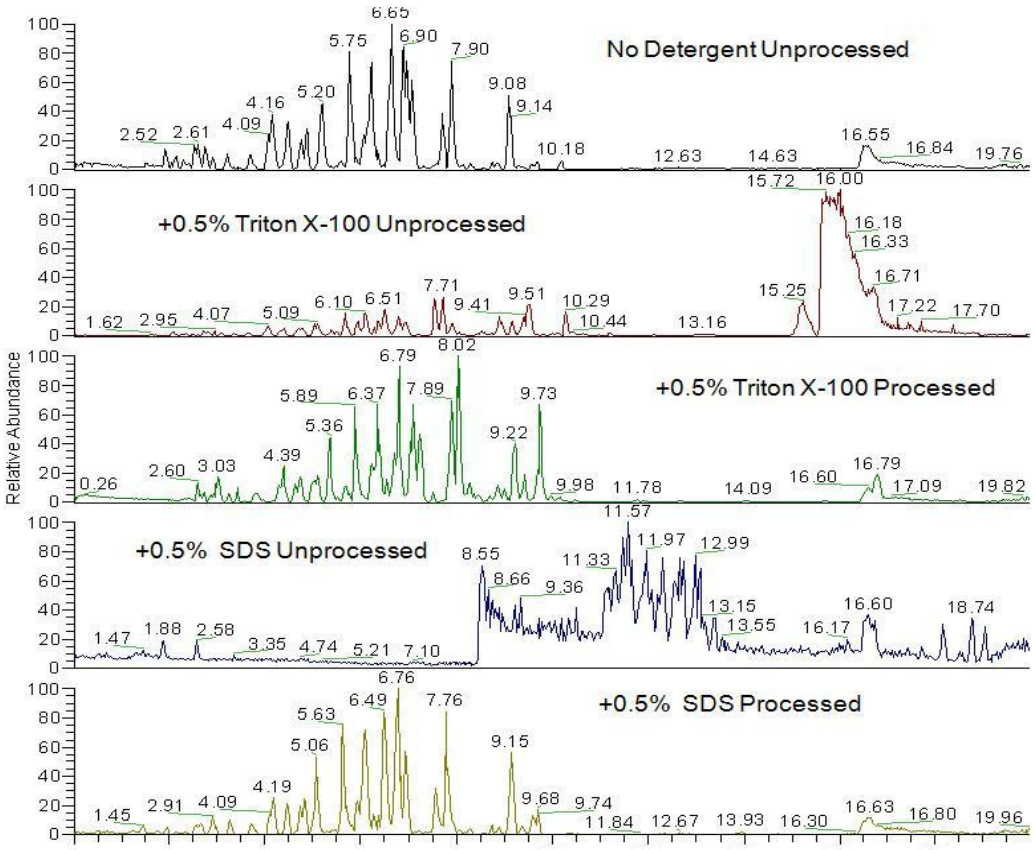


Figure 1. LC-MS/MS analysis of BSA tryptic peptides before and after detergent removal. Base peak LC chromatograms of BSA tryptic peptides. BSA (100µg/mL) in 50mM ammonium bicarbonate buffer, pH 8.0 was reduced, alkylated and digested with trypsin for 3 hours at 37ºC (enzyme-to-protein ratio, 1:50) in the presence of 0.5% detergents. To prepare the sample containing 0.5% SDS, the detergent was added to the sample following tryptic digestion. The 0.1mL digested sample containing the detergent was processed with 0.1mL of Thermo Scientific HiPPR Detergent Removal Resin as shown in the protocol. Control samples (shown above as Unprocessed) were not processed with the detergent removal resin. Samples (3pmol) were loaded directly onto a C18 column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer.

Related Thermo Scientific Products

87776	**Pierce™ Detergent Removal Spin Columns, 125µL,** 25 columns
87777	**Pierce Detergent Removal Spin Columns, 0.5mL,** 25 columns
87778	**Pierce Detergent Removal Spin Columns, 2mL,** 5 columns
87779	**Pierce Detergent Removal Spin Columns, 4mL,** 5 columns
87780	**Pierce Detergent Removal Resin,** 10mL
87782	**Pierce C18 Tips, 10µL bed,** 96/pkg
87784	**Pierce C18 Tips, 100µL bed,** 96/pkg
89870	**Pierce C18 Spin Columns,** 25/pkg
88302	**Pierce Graphite Spin Columns,** 30/pkg
23235	**Micro BCA Protein Assay Kit**

Cited References

1. Rusconi, E., *et al.* (2001). Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal Biochem* **295**:31-7.
2. Urbani, A. and Warne, T. (2005). A colorimetric determination for flycosidic and bile-salt based detergents: applications in membrane protein research. *Anal Biochem* **336**:117-24.

Triton is a trademark of The Dow Chemical Company.

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