INSTRUCTIONS

Pierce® Detergent Removal Spin Columns

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Number	Description
87776	**Pierce Detergent Removal Spin Columns, 125µL,** 25 columns, for 10-25µL samples
87777	**Pierce Detergent Removal Spin Columns, 0.5mL,** 25 columns, for 25-100µL samples
87778	**Pierce Detergent Removal Spin Columns, 2mL,** 5 columns, for 150-500µL samples
87779	**Pierce Detergent Removal Spin Columns, 4mL,** 5 columns, for 500-1000µL samples
87780	**Pierce Detergent Removal Resin,** 10mL

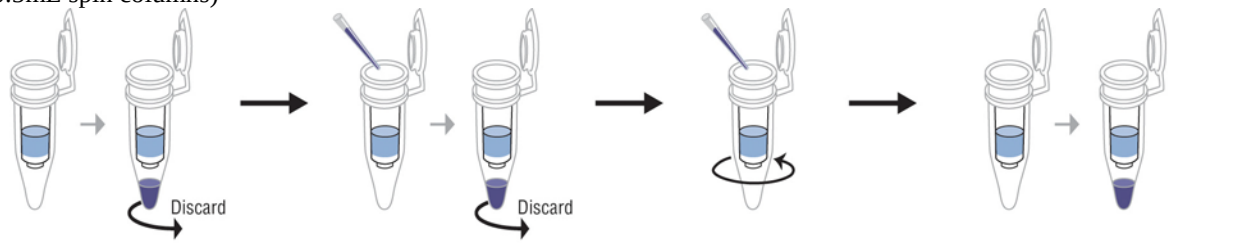
Storage: Upon receipt store at 4°C. Product is shipped at ambient temperature. The resin is stored in 0.15M NaCl, 0.05% sodium azide.

Introduction

The Thermo Scientific Pierce Detergent Removal Resin enables removal of commonly used ionic, non-ionic and zwitterionic detergents from protein/peptide digest solutions. Detergents or surfactants are important for solubilizing, stabilizing and disaggregating protein complexes; however, detergents interfere with downstream analysis including ELISA, isoelectric focusing and mass spectrometry (MS). The presence of detergents causes a deleterious effect by forming adducts with peptides and proteins and, in MS analysis, suppresses peptide ionization and produces a shift in m/z values. Therefore, it is often crucial to remove non-bound detergents from protein digests before downstream analysis. The Pierce Detergent Removal Resin removes detergents with > 95% efficiency at high concentrations (1-5%), while providing exceptionally high protein/peptide recovery for samples > 100µg/mL. For sample concentrations ≤ 100µg/mL, use Thermo Scientific HiPPR Detergent Removal Resin (High Protein and Peptide Recovery).

Procedure Summary

(For 0.5mL spin columns)



1. Centrifuge for 1 minute at 1,500 x *g* to remove the storage buffer.
2. Add 0.4 ml equilibration buffer, centrifuge at 1,500 x *g* for 1 minute and discard the flow-through. Repeat 2 additional times.
3. Add detergent containing sample (25-100 µl) and incubate for 2 minutes at RT.
4. Centrifuge at 1,500 x *g* for 2 minutes to collect the detergent-free sample for downstream applications.

Additional Materials Required

- Variable-speed bench-top microcentrifuge and centrifuge
- 1.5mL and 2mL microcentrifuge collection tubes for the 125µL and 0.5mL spin columns
- 15mL or 50mL conical collection tube for the 2mL or 4mL spin column, respectively
- Wash/equilibration buffer: Use a buffer at pH 4-10, including AMBIC, PBS, MES, MOPS, carbonate bicarbonate or Tris. Do not use buffers containing organic solvents.

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Detergent Removal Procedure

1. Remove bottom closure from column and loosen cap (do not remove cap).
2. Place column into a collection tube (for the 125µL and 0.5mL column use the 2mL collection tube).
3. Centrifuge at the speed and time indicated in Table 1 to remove storage solution. Do not exceed the indicated speed.

   Note: When using fixed-angle rotors, place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced detergent removal efficiency.
4. Add wash/equilibration buffer and centrifuge (Table 1). Discard the buffer. Repeat this step two additional times.
5. Place column in a new collection tube (for the 125µL and 0.5mL column use the 1.5mL collection tube).
6. Slowly apply sample volume to the top of the compact resin bed and incubate for 2 minutes at room temperature.
7. Centrifuge at the indicated speed for 2 minutes to collect the detergent-free sample. Discard the used column.

Table 1. Sample and wash buffer volumes and centrifugation speed requirements.

Column Size		**125µL**	**0.5mL**	**2mL**	**4mL**
Sample Volume Range (µL)		10-25	25-100	150-500	500-1000
Wash/Equilibration Buffer Volume		100µL	400µL	2mL	4mL
Centrifuge Speed (× *g*)		1000	1500	1000	1000
Centrifugation Time (min)	Storage Solution Removal	1	1	2	2
	Washes	1	1	2	2
	Sample Recovery*	2	2	2	2

*Incubate at room temperature for 2 minutes after loading the detergent-containing sample on top of the compacted resin.

Troubleshooting

Problem	Possible Cause	Solution
Sample or buffer does not flow through the resin	Centrifugation problem or exceeded the indicated speed	Ensure that centrifuge is in working properly; do not exceed indicated speed
	Bottom closure was not removed	Ensure bottom closure is removed
Sample contamination	Improper sample loading	Load sample directly to the top of the resin bed; carefully touch pipette tip to resin to expel all sample
	Resin was allowed to dry	Wash with buffer (see sample column preparation)
Low protein/peptide recovery	Protein/peptide concentration was too low (< 0.1mg/mL)	Use HiPPR Detergent Removal Resin

Additional Information

Protein samples containing a wide range of detergents were processed with the Pierce Detergent Removal Resin. Detergents at concentrations from 1 to 5% were effectively removed with generally > 90% protein recovery (Table 2). To demonstrate the high recovery of peptides using the Pierce Detergent Removal Resin, we performed tryptic digests on BSA samples containing a variety of detergents. Detergent removal eliminated interference and allowed high sequence coverage in LC-MS/MS analysis (Table 3).

Table 2. Detergent removal efficiency and protein recovery.

Detergent	Starting Concentration (%)	Detergent Removal (%)	BSA Recovery (%)
SDS	2.5	99	95
Sodium deoxycholate	5	99	100
CHAPS	3	99	90
Octyl glucoside	5	99	90
Octyl thioglucoside	5	99	95
Lauryl maltoside	1	98	99
Triton® X-100	2	99	87
Triton X-114	2	95	100
NP-40	1	95	91
Brij®-35	1	99	97
Tween®-20	0.25	99	87

Samples (0.1mL) containing 1mg/mL of BSA and detergent were processed through 0.5mL of Pierce Detergent Removal Resin as described in the protocol. Residual SDS was measured using Stains-All (Sigma Aldrich);[1] Triton X-100, Triton X-114 and NP-40 were measured by absorbance at 275nm (protein absorbance was subtracted); and sodium deoxycholate, CHAPS, octyl glucoside, octyl thioglucoside and lauryl maltoside were measured by using concentrated sulfuric acid and phenol.[2] Removal of Brij-35 and Tween-20 was monitored by LC-MS/MS and MALDI-MS analysis. Protein concentration was determined with the Thermo Scientific Pierce BCA Protein Assay.

Table 3. LC-MS/MS analysis of BSA tryptic peptides.

Detergent	Processed	Number of Unique Peptides	Sequence Coverage (%)	Number of Total Spectra
No Detergent	No	30	52.2	166
CHAPS	No	2	5.6	5
CHAPS	Yes	33	57.3	182
Lauryl Maltoside	No	21	46.6	62
Lauryl Maltoside	Yes	33	58.6	182
OG	No	0	0	0
OG	Yes	34	57.8	179
OTG	No	0	0	0
OTG	Yes	39	64	173
NP-40	No	25	53.4	70
NP-40	Yes	34	59.8	192
Triton X-114	No	22	43.7	115
Triton X-114	Yes	36	60.6	187
Sodium deoxycholate	No	2	4.45	8
Sodium deoxycholate	Yes	40	64.6	192
SDS	No	7	14.8	11
SDS	Yes	32	56	154

BSA (1mg/mL) in 50mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin for 12 hours at 37ºC (enzyme-to-protein ratio, 1:50) in the presence of 1% of each detergent except SDS, which was added after trypsin digestion. The digested sample (0.1mL) was processed (yes) through 0.5mL Pierce Detergent Removal Column or not processed (no). Samples were diluted and loaded (1.5pmol) directly onto a C_{18} column and subjected to LC-MS/MS analysis using a Thermo Scientific LTQ Mass Spectrometer. No trapping column was used. All data were analyzed using MASCOT (Matrix Science) and Scaffold (Proteome Software).

Related Thermo Scientific Products

88305	**HiPPR Detergent Removal Spin Column Kit**
88306	**HiPPR Detergent Removal Spin Columns, 0.1mL,** 24/pkg
88307	**HiPPR Detergent Removal 96-well Spin Plates, 0.1mL,** 2 plates
20308	**SDS-Out SDS Precipitation Kit**
23225	**Pierce BCA Protein Assay Kit**
22660	**Pierce 660nm Protein Assay Reagent**
22663	**Ionic Detergent Compatibility Reagent**, 5 × 1g

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 glycosidic and bile-salt based detergents: applications in membrane protein research. *Anal Biochem* **336:**117-24.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.