Pierce™ Detergent Removal Spin Columns

Catalog Numbers 87776, 87777, 87778, 87779, 87780

Doc. Part No. 2162164 Pub. No. MAN0011676 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Thermo Scientific™ Pierce™ Detergent Removal Resin enables removal of commonly used ionic, nonionic, 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 >100 µg/mL. For sample concentrations ≤100 µg/mL, use Thermo Scientific™ HiPPR™ Detergent Removal Resin (High Protein and Peptide Recovery).

Contents and storage

Item	Cat. No.	Amount	Storage
Pierce™ Detergent Removal Spin Columns	87776	25 x 125 µL columns (for 10–25 µL samples)	4°C
	87777	25 x 0.5 mL columns (for 25–100 µL samples)	
	87778	5 x 2 mL columns (for 150–500 µL samples)	
	87779	5 x 4 mL columns (for 500–1,000 µL samples)	
Pierce™ Detergent Removal Resin	87780	10 mL[1]	

[1] The resin is stored in 0.15 M NaCl, 0.05% sodium azide.

Workflow

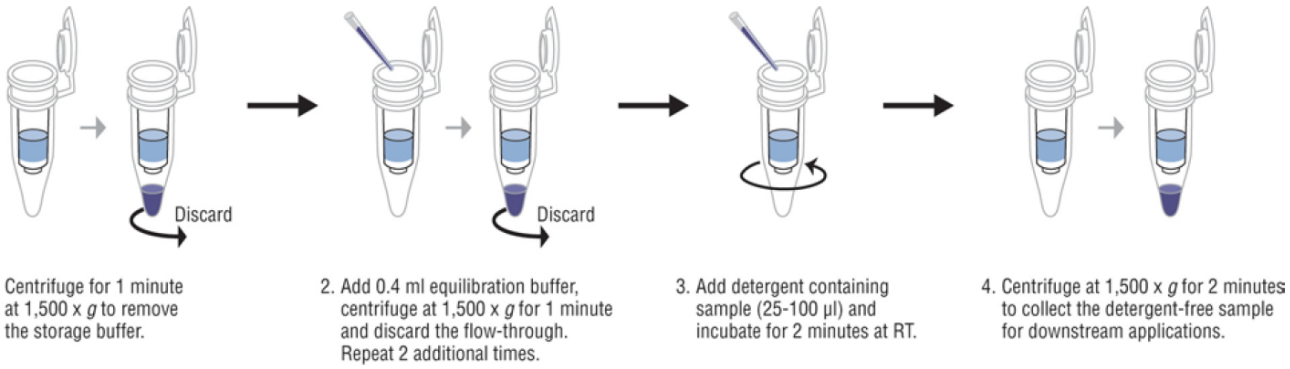

Figure 1 Procedure summary (for the 0.5-mL spin columns).

Required materials not supplied

- Variable-speed bench-top microcentrifuge and centrifuge
- 1.5-mL and 2-mL microcentrifuge collection tubes for the 125-µL and 0.5-mL spin columns
- 15-mL or 50-mL conical collection tube for the 2-mL or 4-mL 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.

Remove detergent from samples

1. Remove the bottom closure from the column and loosen the cap (do not remove the cap).
2. Place the column into a collection tube (for the 125-µL and 0.5-mL column, use the 2-mL collection tube).
3. Centrifuge at the indicated speed and time to remove the storage solution (see Table 1). 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 the column in the centrifuge with the mark facing outward in all subsequent centrifugation steps. Improper orientation will result in reduced detergent removal efficiency.
4. Add the wash/equilibration buffer and centrifuge (see Table 1). Discard the buffer. Repeat 2 additional times.
5. Place the column in a new collection tube (for the 125-µL and 0.5-mL column, use the 1.5-mL collection tube).
6. Slowly apply the 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 (see Table 1). Discard the used column.

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

Column size	Sample volume range	Wash/Equilibration buffer volume	Centrifuge speed	Centrifugation time		
				Storage solution removal	Washes	Sample recovery[1]
125 µL	10–25 µL	100 µL	1,000 x *g*	1 min	1 min	2 min
0.5 mL	25–100 µL	400 µL	1,500 x *g*	1 min	1 min	2 min
2 mL	150–500 µL	2 mL	1,000 x *g*	2 min	2 min	2 min
4 mL	500–1,000 µL	4 mL	1,000 x *g*	2 min	2 min	2 min

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

Troubleshooting

Observation	Possible cause	Recommended action
Sample or buffer does not flow through the resin	Centrifugation problem or exceeded the indicated speed.	Ensure that the centrifuge is working properly; do not exceed indicated speed.
	Bottom closure was not removed.	Ensure that the bottom closure is removed.
Sample contamination	Improper sample loading.	Load the 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 step 4).
Low protein/peptide recovery	Protein/peptide concentration was too low (<0.1 mg/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.

Samples (0.1 mL) containing 1 mg/mL of BSA and detergent were processed through 0.5 mL of Pierce™ Detergent Removal Resin as described in the protocol. Residual SDS was measured using Stains-All (Sigma-Aldrich™) (Rusconi et al., 2001); Triton™ X-100, Triton-X™-114, and NP-40 were measured by absorbance at 275 nm (protein absorbance was subtracted); and sodium deoxycholate, CHAPS, octyl glucoside, octyl thioglucoside, and lauryl maltoside were measured by using concentrated sulfuric acid and phenol (Urbani and Warne, 2005). 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.

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

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

BSA (1 mg/mL) in 50 mM ammonium bicarbonate buffer, pH 8.0 was digested with trypsin for 12 hours at 37°C (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 (0.1 mL) was processed (yes) through 0.5 mL Pierce™ Detergent Removal Spin Columns or not processed (no). Samples were diluted and loaded (1.5 pmol) 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).

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
Octyl glucoside	No	0	0	0

Detergent	Processed	Number of unique peptides	Sequence coverage (%)	Number of total spectra
Octyl glucoside	Yes	34	57.8	179
Octyl thioglucoside	No	0	0	0
Octyl thioglucoside	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

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References

Rusconi E et al. (2001) Quantitation of sodium dodecyl sulfate in microliter-volume biochemical samples by visible light spectroscopy. *Anal Biochem* 295:31–37.

Urbani A, Warne T. (2005) A colorimetric determination for glycosidic and bile-salt based detergents: applications in membrane protein research. *Anal Biochem* 336:117–124.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0011676

Revision	Date	Description
B.0	7 December 2022	The content and format were updated.
A.0	17 October 2015	New document for the Pierce™ Detergent Removal Spin Columns.