DetergentOUT™ GBS10 Spin Plates

96-Well Plates for the Removal of Detergents from Peptide & Protein Solutions

(Cat. # 786-998)

INTRODUCTION

Detergents are essential for protein solubility during protein extraction and sample preparation, especially when working with hydrophobic proteins. The presence of high concentrations of detergents in protein samples can impair ELISA, IEF, protease digestion of proteins and suppress peptide ionization when analyzed by mass spectrometry.

Our DetergentOUT™ GBS10 resin removes free, unbound anionic, nonionic or zwitterionic detergents (e.g. SDS, Triton® X-100 or CHAPS) from aqueous protein and peptide samples with minimal sample loss for downstream analysis. DetergentOUT™ GBS10 has a high binding capacity for detergents, with 6mg SDS for every ml settled resin and 14 mg Triton® X-100 for every ml settled resin.

The spin plate formats allows for multiple sample processing of samples from 25-100µl. Detergent removal from 96 samples can be performed in <15 minutes and samples are suitable for a range of downstream applications. Applications include, but are not limited to, ELISA, IEF, mass spectrometry and NMR.

ITEMS SUPPLIED

Cat. #	Description	Size	Wash/Collection Plates
786-998	DetergentOUT™ GBS-10 Spin Plate	2 plates	4 plates

STORAGE CONDITIONS

The plates are shipped at ambient temperature. Upon arrival, store at 4˚C. If stored and handled correctly the plates have a shelf-life of 1 year.

ADDITIONAL ITEMS NEEDED

- Variable speed centrifuge with rotor and carriers capable of handling stacked plates (4.5cm height) at 500xg or a vacuum manifold.
- Multi-channel pipettor and tips
- Protein/Peptide solution in an aqueous buffer
- Equilibration Buffer: Any aqueous buffer, pH6.5-8.0

BINDING CAPACITY

- SDS (Sodium Dodecyl Sulfate): ~6mg SDS/ml settled resin
- Triton® X-100: ~14mg Triton® X-100/ml settled resin

IMPORTANT INFORMATION

- Plates are compatible with variable speed centrifuges with rotors and carriers capable of handling stacked plates. Use speed of 500-1,000xg with a maximum of 1,000xg.

- Ensure the spin plates are balanced throughout all centrifugations with a duplicate plate filled with an appropriate volume of water.

Sample Load Volume

The recommended load volumes (25-100µl) are a guideline. The actual volumes used will be dependent on your sample, the concentration of detergents to be removed and the recovered purity desired. For optimal removal of detergents, we recommend using a sample volume of <20% of the resin bed volume.

NOTE: *Loading more than the recommended load volume will result in a higher level of contaminating detergents.*

NOTE: *To process >96 samples, evenly divide samples between 2 plates.*

PROTOCOL

1. Equilibrate the DetergentOUT™ Spin Plate to room temperature.
2. Remove the seal from the bottom of the plate and place on top of a wash/collection plate.
3. Remove the seal from the top of the plate.
4. Place the plate assembly in a centrifuge with a 96-well plate carrier and centrifuge at 1,000xg for 1 minute to remove the storage buffer. Discard the storage buffer.
5. Add 300µl of Equilibration Buffer to the resin bed. Centrifuge at 1,000x g for 1 minute and discard the flow-through. Repeat this step once.
6. Rinse the wash plate with deionized water, dry and save for future use.
7. Place the detergent removal plate on a new wash/collection plate and apply 25-100µl sample to the center of the resin.
   NOTE: *Touch the tip to the resin to expel all the sample.*
8. Incubate at room temperature for 2 minutes.
9. Place the plate assembly in a centrifuge with a 96-well plate carrier and centrifuge at 1,000xg for 2 minutes to collect the detergent free sample.
   NOTE: *Discard the DetergentOUT™ plate or save for future use as a balance blank.*

TROUBLESHOOTING

Issue	Reason	Possible Solution
Detergent present in flow through (leaching)	Sample exceeds capacity of resin	Use less sample or a larger format DetergentOUT™ GBS10
	Detergent bound to protein/ peptides	DetergentOUT™ GBS10 only removes free, unbound detergent
No detergent removal	Sample is in non - aqueous solution	If possible perform a buffer exchange by dialysis or use our SpinOUT™ desalting columns.
Peptide/ Protein Loss	Protein sample too dilute	Concentrate peptide/protein solution, or use less DetergentOUT™ GBS10
	Resin: Peptide/Protein Solution Ratio too high	Reduce the volume of DetergentOUT™ GBS10 used
Solutions not passing through the columns	Resin compression due to high centrifugal forces	Reduce centrifugal force and centrifuge times
		Perform entire procedure in a gravity flow manor

APPENDIX 1: DETERGENT REMOVAL EFFICIENCY FROM PROTEIN SOLUTIONS

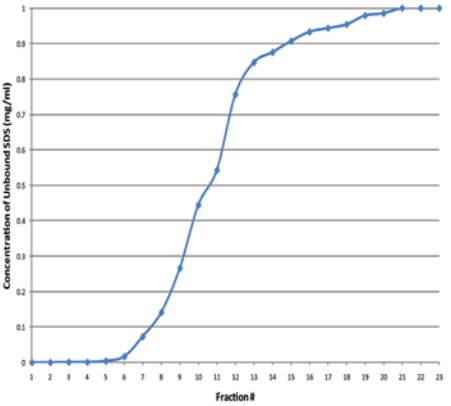

Figure 1: DetergentOUT™ GB-S10 retains ≤6mg SDS per ml settled resin. 2ml DetergentOUT™ GB-S10 resin was pipetted into an appropriate column and was washed with Equilibration Buffer as indicated in the protocol. To monitor SDS binding capacity, 50ml 0.1% (1mg/ml) SDS solution was continuously applied to the column. 2ml fractions were collected and assayed for the presence of SDS, using our SDS assay. The graph depicts the amount of SDS detected in the flow-through, i.e. not retained by the column. The graph shows that SDS was not detected until fraction 7, so after 12mg SDS had been retained by the 2ml of DetergentOUT™ GB-S10 resin, resulting in a 6mg/ml settled resin binding capacity.

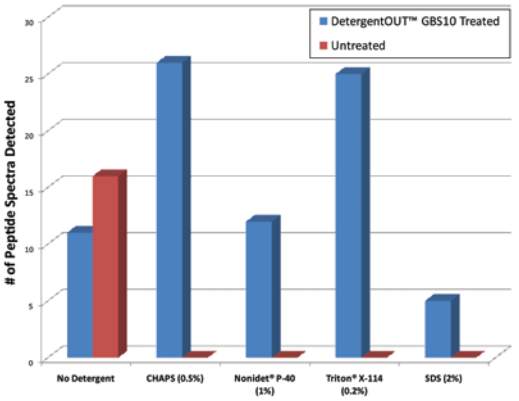

Figure 2: DetergentOUT™ GBS10 removes detergent and allows detection of peptide fragments by Mass spectrometry[1]. 500µg phosphorylase B was digested in solution and then the indicated amount of detergent was added. Samples were treated with DetergentOUT™ GBS10 according to this protocol. Samples were resuspended in 5% ACN/ 0.1% FA, ziptipped using C18, and infused using nanospray tips into an ABI QSTAR XL (Applied Biosystems/ MDS Sciex) hybrid QTOF MS/MS mass spectrometer. TOF mass and product ion spectra were acquired using information dependent data acquisition (IDA) in Analyst QS v1.1 with the following parameters: mass ranges for TOF MS and MS/MS were m/z 300-2000 and 70-2000, respectively. Every second, a TOF MS precursor ion spectrum was accumulated, followed by three product ion spectra, each for 3 s. *(Alvarez, S. et al)*

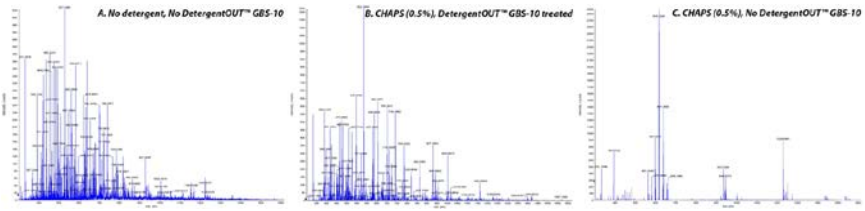

Figure 3: DetergentOUT™ GBS10 removes CHAPS and enhances Mass spectrometry Spectra[1]. 5µg/µl protein mixture (BSA, cyctochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 according to this protocol and compared to an untreated sample (Panel C). Samples were resuspended in 5% ACN/ 0.1% FA, ziptipped using C18, and infused using nanospray tips into an ABI QSTAR XL (Applied Biosystems/MDS Sciex) hybrid QTOF MS/MS mass spectrometer. TOF mass and product ion spectra were acquired using information dependent data acquisition (IDA) in Analyst QS v1.1 with the following parameters: mass ranges for TOF MS and MS/MS were m/z 300-2000 and 70-2000, respectively. Every second, a TOF MS precursor ion spectrum was accumulated, followed by three product ion spectra, each for 3 s. *(Alvarez, S. et al)*

Spin columns containing 0.5ml DetergentOUT™ GBS10 resin were prepared and processed according to the protocol. 0.1ml 1mg/ml protein solutions supplemented with 1-5% detergent were processed. The DetergentOUT™ GBS10 resin effectively removed detergents with >90% protein recovery.

Detergent	% Removed	Total Protein Recovery			
		BSA	Phosphorylase B	Cytochrome C	E. coli Lysate
Triton® X-100, 2%	>99%	>90%	>91%	>92%	>93%
Triton® X-114, 2%	>96%	>99%	>98%	>97%	>91%
Nonidet® P-40, 1%	>96%	>93%	>95%	>91%	>91%
Brij® 35, 1%	>99%	>98%	>99%	>97%	>91%
SDS, 2.5%	>99%	>96%	>97%	>92%	>90%
Sodium deoxycholate, 5%	>99%	>99%	>99%	>98%	>95%
CHAPS, 3%	>99%	>92%	>95%	>92%	>91%
Octyl glucoside, 5%	>99%	>93%	>95%	>96%	>91%
Lauryl maltoside, 1%	>97%	>99%	>99%	>99%	>91%
Tween® 20, 0.25%	>98%	>86%	>85%	>89%	>85%
Tween® 80, 0.13%	>85%	>83%	>81%	>80%	>81%

Table 1: A comparison of the detergent removal rates and percentage protein recovery with DetergentOUT™ GBS-10.

REFERENCES

Alvarez, S. et al. Efficiency assay of detergent removal columns on protein and peptide samples for mass spectrometric analysis. Poster presented as part of the 58th ASMS Conference on Mass Spectrometry and Allied Topics, May 23-27, 2010, Salt Lake City, Utah

RELATED PRODUCTS

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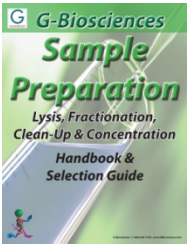

http://info.gbiosciences.com/complete-protein-sample-preparation-handbook/

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