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A Geno Technology, Inc. (USA) brand name

DetergentOUT™ Detergent Removal Systems

For the Removal of Detergents from Peptide & Protein Solutions

G-Biosciences offers three detergent removal systems. DetergentOUT™ GBS10 features a detergent binding resin that has high affinity for most detergents and a low affinity for proteins and peptides. DetergentOUT™ GBS10 will meet most researcher requirements and is ideal for detergent removal prior to ELISA, IEF, protease digestion of proteins and peptide ionization when analyzed by mass spectrometry.

DetergentOUT™ Tween® is a detergent binding resin that offers higher binding affinity for polysorbate detergents, commercially known as Tween®. The DetergentOUT™ Tween® resin binds other commonly used detergents, but with lower affinity compared to DetergentOUT™ GBS10.

OrgoSol DetergentOUT™ is suitable for removal of detergents from protein solutions, including hydrophobic protein solutions. OrgoSol DetergentOUT™ uses protein precipitation followed by washing to remove the detergents. The protein pellet is then reconstituted in a buffer of choice. Protein precipitation may result in loss of the protein's biological activity. OrgoSol Detergent*OUT*™ is compatible with all detergent types and its performance is not dependent on the concentration of detergents in the solution. OrgoSol Detergent*OUT*™ is highly flexible and can be used to process small and large sample volumes.

DetergentOUT™ GBS10

DetergentOUT™ Tween®

OrgoSol DetergentOUT™

DetergentOUT™ GBS10

For the Removal of Detergents from Peptide & Protein Solutions

KIT COMPONENTS

Cat. #	Description	Sample Size (µl)	Resin Volume (µl)	Size
786-154	DetergentOUT™ GBS10-125	10-30	125	10 columns
786-155	DetergentOUT™ GBS10-800	30-200	800	10 columns
786-156	DetergentOUT™ GBS10-3000	200-750	3,000	10 columns
786-157	DetergentOUT™ GBS10-5000	500-1,250	5,000	10 columns
786-159	DetergentOUT™ GBS10 Resin	-	-	10ml resin

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.

STORAGE CONDITIONS

Shipped at ambient temperature. Store at 4°C, do not freeze.

ADDITIONAL ITEMS NEEDED

- Collection tubes
- 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

PREPARATION BEFORE USE

1. Prior to use, spin the DetergentOUT™ GBS10 columns with the top and then bottom caps in place at 1,000xg for 1 minute to pellet the resin.
2. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.

PROCEDURE: DetergentOUT™ GBS10-125 for 10-30µl sample

1. Remove the bottom then top cap of the column. Place the column in a 2ml collection tube and centrifuge for 1 minute at 1,000xg to remove the storage buffer.
2. Add 100µl Equilibration Buffer and then centrifuge for 1 minute at 1,000xg. Discard the flow-through.
3. Repeat the equilibration step once.
4. Add 10-30µl detergent containing, aqueous protein/peptide solution, followed by 3µl Equilibration Buffer and incubate at room temperature for 2 minutes.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 1,000xg to collect the detergent-free sample.

PROCEDURE: DetergentOUT™ GBS10-800 for 30-200µl sample

1. Snap off the bottom of the column and remove the cap. Place the column in a 2ml collection tube and centrifuge for 1 minute at 1,500xg to remove the storage buffer.
2. Add 400µl Equilibration Buffer and then centrifuge for 1 minute at 1,500xg. Discard the flow-through.
3. Repeat the equilibration step once.

4. Add 30-200µl detergent containing, aqueous protein/peptide solution and incubate at room temperature for 2 minutes.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 1,500xg to collect the detergent-free sample.

PROCEDURE: DetergentOUT™ GBS10-3000 for 200-750µl sample

1. Remove the bottom then top cap of the column. Place the column in a 15ml collection tube and centrifuge for 2 minutes at 1,500xg to remove the storage buffer.
2. Add 2ml Equilibration Buffer and then centrifuge for 2 minutes at 2,000xg. Discard the flow-through.
3. Repeat the equilibration step once.
4. Add 200-750µl detergent containing, aqueous protein/peptide solution and incubate at room temperature for 2 minutes.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 2,000xg to collect the detergent-free sample.

PROCEDURE: DetergentOUT™ GBS10-5000 for 500-1,250µl sample

1. Remove the bottom then top cap of the column. Place the column in a 15ml collection tube and centrifuge for 2 minutes at 1,500xg to remove the storage buffer.
2. Add 4ml Equilibration Buffer and then centrifuge for 2 minutes at 2,000xg. Discard the flow-through.
3. Repeat the equilibration step once.
4. Add 500-1,250µl detergent containing, aqueous protein/peptide solution and incubate at room temperature for 2 minutes.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 2,000xg to collect the detergent-free sample.

PROCEDURE: DetergentOUT™ GBS10 Resin

1. The resin can be aliquoted into a spin column format or used in a batch format by adding resin direct to aqueous peptide or protein solutions.
2. First aliquot an appropriate volume of DetergentOUT™ GBS10 resin into a column or centrifuge tube. Centrifuge at 2,000xg to spin out storage buffer of pellet resin. Discard the storage buffer.

   NOTE: We recommend using ≥4ml settled resin for every 1ml protein/peptide solution. Optimization of the resin: sample ratio may improve the efficiency of detergent removal and recovery of protein/peptide.
3. Wash the resin two times with 1-2 resin volumes of Equilibration Buffer.
4. Add the peptide/ protein solution and incubate for 2 minutes at room temperature. If using batch format then incubate with mixing.
5. Centrifuge for 2 minutes at 2,000xg to collect the detergent-free sample (the flow through or supernatant).

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

APPENDIX 1: DetergentOUT™ GBS10 Detergent Removal Efficiency from Protein Solutions

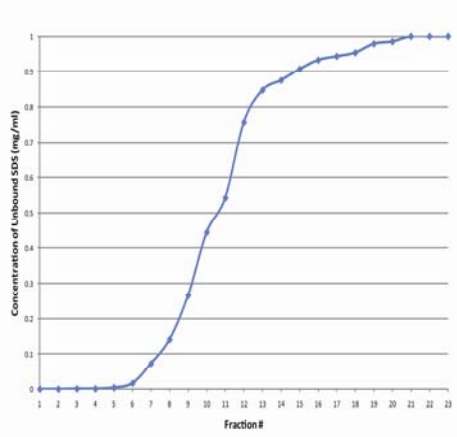

Figure 1: DetergentOUT™ GB-S10 retains ≤6mg SDS per ml settled resin. 2ml DetergentOUT™ GB-S10 resin was pipetted into an appropriate column and was washed with Equilibration Buffer as indicated in the protocol. To monitor SDS binding capacity, 50ml 0.1% (1mg/ml) SDS solution was continuously applied 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.

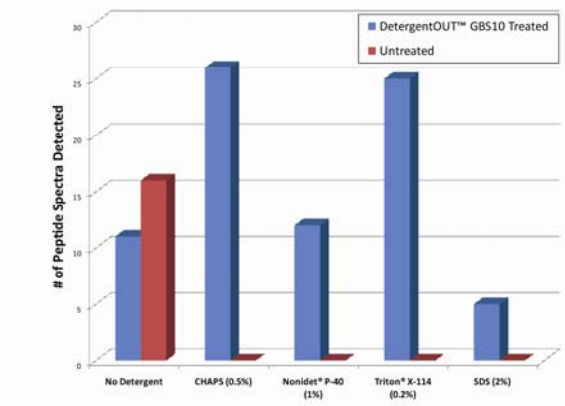

Figure 2: DetergentOUT™ GBS10 removes detergent and allows detection of peptide fragments by Mass spectrometry[1]. 500µg phosphorylase B was digested in solution and then the indicated amount of detergent was added. Samples were treated with DetergentOUT™ GBS10 according to this protocol. Samples were 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)*

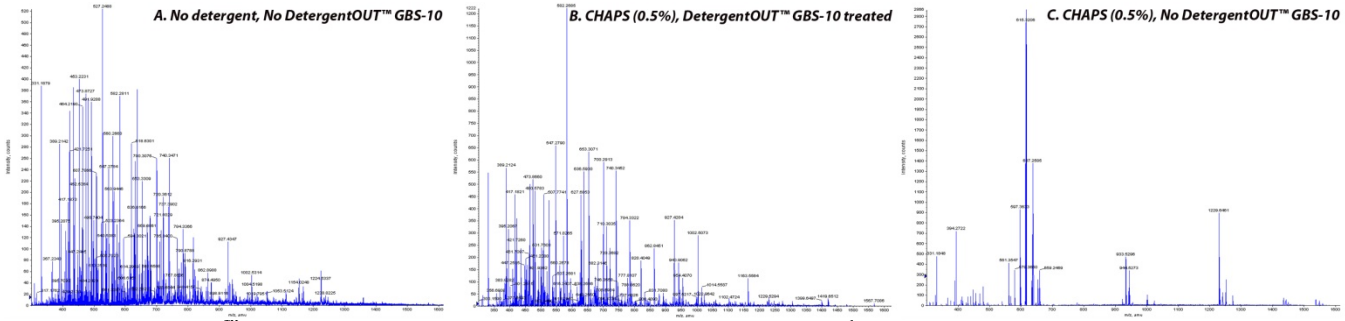

Figure 3: DetergentOUT™ GBS10 removes CHAPS and enhances Mass spectrometry Spectra[1]. 5µg/µl protein mixture (BSA, cyctochrome C and phosphorylase B) in water (Panel A) was supplemented with 0.5% CHAPS (Panel B and C). The CHAPS containing sample was treated with DetergentOUT™ GBS10 according to this 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.

		Total Protein Recovery			
Detergent	**% Removed**	**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

1. 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

Detergent-*OUT*™ Tween®

For Removal of Polysorbate (Tween®) Detergents

KIT COMPONENTS

Cat. #	Description	Sample Size (µl)	Resin Volume (µl)	Size
786-214	DetergentOUT™ Tween® (Micro)	30-500	800	10 columns
786-215	DetergentOUT™ Tween® (Medi)	500-2,000	3,000	10 columns

INTRODUCTION

The Detergent-*OUT*™ Tween® resin has a high binding affinity for polysorbate detergents, commercially known as Tween®. The DetergentOUT™ Tween® resin binds other commonly used detergents, but with lower affinity compared to DetergentOUT™ GBS10.

STORAGE CONDITIONS

Shipped at ambient temperature. Store at 4°C, do not freeze.

ADDITIONAL ITEMS NEEDED

- Collection tubes
- Equilibration Buffer (Deionized water)

PREPARATION BEFORE USE

1. Prior to use, spin the DetergentOUT™ Tween® columns with the top and then bottom caps in place at 1,000xg for 1 minute to pellet the resin.
2. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.

PROCEDURE: DetergentOUT™ Tween® (Micro) for 30-500µl sample

1. Snap off the bottom of the column and remove the cap. Place the column in a supplied 2ml collection tube and centrifuge for 1 minute at 1,500xg to remove the storage buffer.
2. Add 500µl Equilibration Buffer and then centrifuge for 1 minute at 1,500xg. Discard the flow-through.

   NOTE: Detergent-OUT™ columns are supplied in deionized water. For optimal polysorbate detergent removal, equilibrate the column with deionized water. Columns may be equilibrated with other types of buffers, however, do not equilibrate the columns with high concentration (>50mM) organic buffers, such as Tris buffer and other organic buffers as these may reduce the detergent binding capacity.
3. Repeat the equilibration step two more times.
4. Add 30-500µl polysorbate detergent containing, aqueous protein/peptide solution and incubate at room temperature for 10-15 minutes, reload any flow through.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 1,500xg to collect the detergent-free sample.

PROCEDURE: DetergentOUT™ Tween® (Medi) for 500-2,000µl sample

1. Remove the bottom then top cap of the column. Place the column in a 15ml collection tube and centrifuge for 3 minutes at 1,000xg to remove the storage buffer.
2. Add 2ml Equilibration Buffer and then centrifuge for 2 minutes at 1,000xg. Discard the flow-through.

   NOTE: Detergent-OUT™ columns are supplied in deionized water. For optimal polysorbate detergent removal, equilibrate the column with deionized water. Columns may be equilibrated with other types of buffers, however, do not equilibrate the columns with high concentration (>50mM) organic buffers, such as Tris buffer and other organic buffers as these may reduce the detergent binding capacity.
3. Repeat the equilibration step two more times.
4. Add 500-2,000µl detergent containing, aqueous protein/peptide solution and incubate at room temperature for 2 minutes.
5. Place column into a clean collection tube, centrifuge for 2 minutes at 1,500xg to collect the detergent-free sample.

OrgoSol DetergentOUT™

Concentrate and Remove Detergents from Protein Solution

KIT COMPONENTS

Cat. #	786-127 (Micro)	786-128 (Medi)
UPPA-I	30ml	100ml
UPPA-II	30ml	100ml
OrgoSol Buffer	50ml	2 x 50ml
DO Wash	2.0ml	2 x 2.0ml
SEED	300µl	2 x 300µl
DO Prep Buffer-I	2.0ml	2 x 2.0ml
DO Prep Buffer-II	0.5ml	2 x 0.5ml

INTRODUCTION

The column-based method of removing detergents is simple to use and compared to the resin based detergent removal systems has no limited binding capacity and is suitable for hydrophobic proteins. The *OrgoSol* DetergentOUT™ kit has been specifically developed for removing all types of detergent from protein solution. *OrgoSol* DetergentOUT™ can be used for removing ionic, non-ionic and cationic detergents and also suitable for removing detergents from hydrophobic proteins. This kit is based on protein precipitation, followed by removal of detergent from the protein pellet. After removing detergent, the protein pellet is suspended in buffer of choice.

The *OrgoSol* DetergentOUT™ kit is supplied in two sizes: the Micro Kit is for removing detergents from up to a total of 10ml dilute protein solution, either single or multiple procedures, and the Medi Kit is for removing detergents from up to a total of 30ml dilute protein solution, either single or multiple procedures. Additional volumes of any reagent may be purchased separately.

OrgoSol DetergentOUT™ is ideal for the removal and concentration of protein solutions for isoelectric focusing, 2D gels, raising antibodies, electrophoresis, protein assays, and other applications.

STORAGE CONDITIONS

Shipped at ambient temperature. Store at room temperature upon arrival. The OrgoSol Buffer must be pre-chilled to -20°C prior to use.

ADDITIONAL ITEMS NEEDED

- Collection tubes
- Spin columns

PREPARATION BEFORE USE

1. Prior to use, chill the OrgoSol Buffer at -20°C for at least 1 hour prior to use.

PROCEDURE: OrgoSol DetergentOUT™ (Micro)

NOTE: Perform the entire procedure in the cold, on ice, unless specified otherwise. Concentration should be performed in a centrifuge tube. For small volumes, use microfuge tubes. Always position microfuge-tubes in the centrifuge at the same orientation, i.e. cap-hinge facing out-ward. This will allow the pellet to remain glued to the same side of the tube during repeated centrifugations and minimize the loss of protein pellets.

1. Add 300µl UPPA-I to every 100µl protein solution. Vortex the mixture and incubate on ice for 10 minutes.

2. Add 300µl UPPA-II for every 100µl original protein solution to the protein/UPPA-I mixture and vortex.

3. Place the tubes in the centrifuge with the lid hinge facing outwards. Centrifuge the tube at 15,000xg for 5 minutes to form a tight pellet.

4. Immediately after centrifugation, carefully and without disturbing the pellet, remove the entire supernatant.

5. Carefully position the tube in the centrifuge as before, i.e. cap-hinge facing outward. Centrifuge the tube for 30

seconds at 15,000xg. Use a pipette tip and remove the remaining supernatant.

6. Add 40µl of DO Wash on top of the pellet (for larger sample sizes, add DO Wash 3-4x times the size of the pellet). Carefully position the tube in the centrifuge as before, i.e. cap-hinge facing outward. Centrifuge the tube for 5 minutes at 15,000xg. Use a pipette tip, remove and discard the wash.

7. Add 25µl of deionized water to the pellet. Vortex the tube.
   NOTE: For larger pellets, add enough water to cover the pellet. The protein pellet will not dissolve in the water.

8. For every 100-300µl initial protein solution, add 1ml pre-chilled OrgoSol Buffer and 5µl SEED.
   NOTE: For larger pellets, use 10X OrgoSol Buffer compared to the water used in step 7.

9. Vortex to suspend the pellet. It is important that the pellet is fully suspended in OrgoSol Buffer. The pellets do not dissolve in OrgoSol Buffer. Incubate the tube at -20^{o}C for 30 minutes. Periodically vortex the tube, 20-30 seconds vortex each burst.

10. Centrifuge at 15,000xg for 5 minutes to form a tight pellet.

11. Remove and discard the supernatant. You will notice a white pellet in the tube. Air-dry the pellet. On drying, the white pellet will turn translucent.
    NOTE: Do not over dry the pellet; parched dry pellets may be difficult to dissolve.

12. Suspend the pellet in an appropriate volume of DO Prep Buffer-I (5-50µl DO Prep Buffer-I). Vortex to suspend the pellet. Incubate for 5minutes.
    NOTE: Samples containing >100µg protein produces large and tightly packed protein pellets, which require a longer time to dissolve in buffers. Grinding of the protein pellet with a pestle will accelerate solubilization of the pellet. We recommend use of microfuge tubes and tight fitting pestle for processing samples containing more than 100µg protein (See related products).

13. Add DO Prep Buffer-II. For each 5µl DO Prep Buffer-I used, add 1µl of DO Prep Buffer-II. Incubate for 5 minutes. After the pellet is dissolved, centrifuge and collect a clear protein solution. The protein solution at this stage contains 60mM Tris, pH 7-8.

14. After dissolving the pellet, the protein solution may be mixed with Urea, Guanidine.HCl, SDS-PAGE gel loading buffer or other types of buffers and agents.

15. For buffer exchange, the protein suspension may be dialyzed or passed through a pre-equilibrated spin column.

RELATED PRODUCTS

1. **SpinOUT™ Columns** (Cat. # 786-170 to 786-173, 786-703 to 786-708). *The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein solutions ranging from 5µl through to 4ml sample volumes.*
2. **Trypsin, Mass Spectrometry Grade** (Cat. # 786-248, 786-687, 786-688). *A chemically methylated to yield an enzymatically active protein with maximum trypsin specificity and is extremely resistant to autolysis. In addition the modified trypsin is TPCK treated to inactive the interfering chymotrypsin activity and the resulting protein is affinity purified and lyophilized. The resulting trypsin is extremely resistant to autolysis and has a specific activity over 10,000 units/mg protein. Our Mass Spectrometry Grade Trypsin is highly stable; maintaining its activity in severe denaturing buffers and as a result can be stored for a long period without any loss of activity.*
3. **InGel™ silver** (Cat. # 786-241) *A reliable method for the proteolytic digestion and subsequent extraction of silver stained proteins in gel for subsequent analysis by mass spectrometry.*
4. **Immobilized Trypsin** (Cat. # 786-792). *A reliable method for the proteolytic digestion and subsequent extraction of silver stained proteins in gel for subsequent analysis by mass spectrometry.*
5. **Pestles & Tubes** (Cat # 786-138P): *For grinding small samples of tissue, cells and protein pellets.*

For related products, visit www.GBiosciences.com.