One-Step Detergent Removal Kit

Introduction

Detergents contain a hydrophilic head group and a hydrophobic tail. The hydrophobic moiety usually consists of a hydrocarbon chain, while the hydrophilic part has a polar head. Three types of detergents are commonly used in laboratory research, including:

- Non-ionic detergents contain uncharged hydrophilic head groups such as Triton X-100, Triton X-114, NP-40, Tween-20, and Tween-80.
- Ionic detergents are comprised of a hydrophobic chain and anionic or cationic head groups such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB).
- Zwitterionic detergents: Those detergents like CHAPS contain negatively and positively charged atomic groups in equal numbers. Therefore, they do not possess a net charge.

Detergents (surfactants) are essential for biomedical research. For successful downstream analysis, it is critical to reduce or entirely remove unbound detergents from the biological sample. However, excess unbound detergent is usually poorly compatible with many downstream applications, including ELISA, protease digestion of proteins, isoelectric focusing, and mass spectrometry (MS). Several commercially available detergent removal methods include prolonged dialysis, anion exchange chromatography, detergent removal spin column, and acetone precipitation. However, these procedures, such as using detergent removal columns, are either laborious or suffer from sample losses and are challenging for low volume samples and high thorough-put automation. We developed a novel, efficient surfactant removal system to overcome these limitations.

BcMag™ One-Step Detergent Removal Kit uses magnetic resin coated with proprietary chemistry to remove detergents. Compared with the detergent removal columns, the resin can quickly and efficiently remove free detergents from the sample with just a single step and enables individual samples or 96 samples to be processed simultaneously in less than 1 minute or 10 minutes with 95% sample recovery. Since the magnetic resin only adsorbs the detergent, the sample recovery rate is exceptional >90%-95%.

Workflow

The detergent removal protocol is straightforward (Fig.1). 1. Add the beads directly to the sample. 2. Pipette or vortex to capture the free SDS detergent. 3. Magnetic separation of the beads from the protein, or DNA/RNA solution, while the protein or DNA/RNA remains in the solution. The easy-to-use magnetic beads significantly improve results over the standard drip column and batch methodologies with minimum protein loss (<10%). Due to using only a small volume of magnetic beads, the final protein concentration of the sample is not significantly decreased.

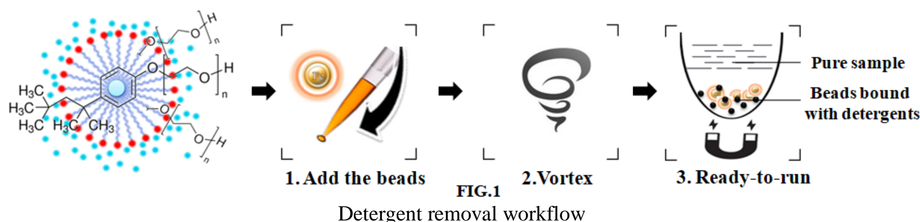

FIG.1
Detergent removal workflow

Features and Advantages

- Simple protocol: No liquid transfer, One-tube, One-step, and one-minute protocol.
- Easy to use.
- Reliable and reproducible results with exceptional >90% recovery for protein (>6 kDa, aprotinin) or DNA/RNA (>25mer dsDNA)
- Effective Cleanup: Remove 95% free detergent.
- Cost-effective: Eliminates columns, filters, and laborious repeat pipetting.
- High throughput: Compatible with many different automated liquid handling systems.

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Specification	
Composition	Silica-enclosed magnetic beads are modified with our proprietary chemistry.
Stability	Short Term (<1 hour): pH 4-11; Long-Term: pH 4-10 Temperature: 4°C -140°C; Most organic solvents
Magnetization	~40-45 EMU/g
Type of Magnetization	Superparamagnetic
Formulation	100 mg / ml in dH2O
Storage	Ship at room temperature, Store at 4° upon receipt.

PROTOCOL

Materials Required by the User

Item	Source
Magnetic rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag magnetic rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag magnetic rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag magnetic rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag magnetic rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag 96-well Plate Magnetic rack.	• BcMag 96-well Plate Magnetic rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** *IMPORTANT!* If using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates must be ≥2.5mm.	
Fisher Scientific™ Microplate Advanced Vortex Mixers	Fisher, Cat#:02-216-101
OHAUS Microplate Vortex Mixers	OHAUS, Cat#:30392160
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be Orbit ≥1.5 mm-4 mm, Speed≥ 800 rpm	
Clear Flat-bottom Non-Binding Assay Microplates	

Procedure

IMPORTANT!

- ***The following protocol is an example. The beads and sample volume can be rational Scale-up (or down). Do not use buffers containing organic solvents.***
- ***The user should optimize the beads and detergent concentration ratio based on the binding capacity listed in table 1.***

Table 1

Detergent	Binding Capacity**	Protein Recovery (%)
Triton* X-100	17 µg/mg beads	>97
Triton X-114	16.5 µg/mg beads	>96

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Tween-20	9 µg/mg beads	>92
NP-40	16 µg/mg beads	>96
Brij-35	16 µg/mg beads	>94
Tween-80	16 µg/mg beads	>93
DDM	15 µg/mg beads	>93
CY-6	16.5 µg/mg beads	>97
CTAB	10 µg/mg beads	>60

** ***Binding capacity assay condition: Mix with 10 µl magnetic beads (100 mg/ml) with 100 µl protein sample (1:400 dilution of Human serum) containing detergents in 0.1M Sodium phosphate, 0.15M NaCl, pH7.5 buffer, and vortex at 2000 rpm for 5 minutes)***

Procedure

1. Shake the bottle to resuspend the Magnetic beads until it is homogeneous entirely.

   IMPORTANT!

   It is essential to mix the beads before dispensing. Do not allow the beads to sit for more than 2 minutes before dispensing. Resuspend the magnetic beads every 2 minutes.
2. Add an appropriate amount of the magnetic beads to the sample containing free detergent. Mix the sample with beads for 1-2 minutes by slowly pipetting up and down 20-25 times *or* vortex for 5 minutes at 2000 rpm for PCR plates or .800 rpm for microplate**s.**

   IMPORTANT!

   - Users need to optimize the beads and free detergents ratio based on the binding capacity listed in table 1.
   - Optimize the Speed and time if using a vortex mixer.
3. Place the sample plate or tube on the magnetic separation plate for 30 seconds or until the solution is clear.
4. Transfer the supernatant to a clean plate /tube while the sample plate remains on the magnetic separation plate. The sample is ready for downstream applications.

C. Troubleshooting

Problem	Probable cause	Suggestion
Low Protein Recovery	Vortexing time is too long.	• If using other digital vortex mixers, the vortex condition such as Speed and time has to be optimized.
	Using too many magnetic beads	Completely resuspend the magnetic beads and reduce the amounts of the beads.
Failure to remove detergent.	Used inappropriate tubes or plates	Ensure that the well diameter at the bottom of the conical section of the Tubes or well of the plate is ≥2.5mm.
	• Vortex speed is too slow, or vortex time is too short. • Containing too much SDS in the sample	• Increasing either the Speed or time • If using other digital vortex mixers, the vortex condition such as Speed and time must be optimized. • Repeat the procedure using more beads

Related Products	
Product Name	Product Name
One-Step Lipids Removal Kit	Quick Albumin Removal Kit
One-Step Deproteinizing Kit	Quick HSA and IgG Depletion Kit
One-Step SDS Removal Kit	One-Step Dye Removal Kit
One-Step Detergent Removal Kit	Quick Endotoxin Removal Kit
EDTA Metal Ion removal Kit	Immobilized TCEP Disulfide Reducing Kit
EGTA Metal Ion removal Kit	One-Step PCR Inhibitor Removal Kit
One-Step DNA and RNA Cleanup Kit	One-Step DNA and RNA Removal Kit
One-Step Sequencing Cleanup Kit	One-Step Single-Stranded DNA Removal Kit
One-Step Fluorescent Labeling Cleanup Kit	One-Step RNA Removal Kit
One-Step NGS Cleanup Kit	One-Step PCR Cleanup Kit

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