

# **One-Step Deproteinizing Kit**

### Introduction

The presence of protein and lipids in biological fluid samples frequently interferes with small molecule analysis, decreasing accuracy and sensitivity. Numerous bioassays need the removal of proteins (deproteinization) and lipids (delipidation) from samples before analysis, such as LC/MS/MS analysis.

Precipitation using trichloroacetic acid, perchloric acid, phosphotungstic acid, acetonitrile, methanol, and other organic solvents has been extensively used for deproteinization over the last half-century. However, these procedures are not only tedious, time-consuming, and challenging to adapt to automation but also inefficiently remove lipids and nucleic acids. **BcMag<sup>TM</sup> One-Step Deproteinizing Kit** provides a novel solution for the deproteinization, delipidation, and nucleic acid from biological fluid samples such as cell lysis, plasma, serum, tissue homogenate, and urine for analysis of a variety of small molecules. The superparamagnetic magnetic beads efficiently utilize the proprietary matrix to bind proteins and lipids in the sample. The one-minute protein removal protocol is straightforward: one step, one tube, no precipitation, no filtration, and no centrifugation. The resin enables 96 samples to be processed simultaneously in less than 10 minutes.

#### Workflow



#### Features and Advantages:

- Fast and straightforward protocol: one step, one tube, process 96 samples in less than 10 minutes
- Easy-to-use: Eliminates columns, filters, laborious repeat pipetting or centrifugation, and easily adjusts for sample size and automation
- No organic solvents/acids precipitation
- Efficient deproteinization

Specification				
Composition	ition Magnetic beads are modified with our proprietary chemistry.			
	Short Term (<1 hour): pH 4-11; Long-Term: pH 4-10			
Stability	Temperature: 4°C -140°C; Most organic solvents			
Magnetization	~40-45 EMU/g			
Type of Magnetization	Superparamagnetic			
Formulation	100 mg / ml in dH <sub>2</sub> O			
Binding Capacity	50 µg proteins/mg of Beads			
Storage	Ship at room temperature, Store at 4° C upon receipt.			

Products	Catalog # BB101	Catalog # BB-102
BcMag <sup>™</sup> One-Step Deproteinizing Kit	1 ml	5 ml

1



# PROTOCOL

### A. 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	<ul> <li>BcMag magnetic rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01)</li> <li>BcMag magnetic rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02)</li> <li>BcMag magnetic rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03)</li> <li>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)</li> </ul>				
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 (Bioclone, 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 <sup>TM</sup> MixMate <sup>TM</sup>	Eppendorf, Cat#:5353000529				
Tube Holder PCR 96	Eppendorf, Cat#: 022674005				
Tube Holder $1.5/2.0$ mL, for $24 \times 1.5$ 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 ** <i>IMPORTANT</i> ! 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 <sup>TM</sup> 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 Elat-bottom Non-Binding Assay Microplates					

# **B.** Procedure

Notes:

- Do not use buffers containing organic solvents.
- For the best results, ensure that the sample pH should be ~4.5.
  - 1. Dilute and adjust the protein concentration in sample ~1mg/ml and pH to 4.5 by sodium acetate or other buffers.
  - 2. Shake the bottle to resuspend the Magnetic Beads until it is homogeneous completely.

## **IMPORTANT!**

# • Resuspend the magnetic Beads every 2 minutes. It is essential to mix the Beads before dispensing. Do not allow the Beads to sit for more than 2 minutes before dispensing.

- Add an appropriate amount of the magnetic Beads to the sample (Beads amount should be calculated based on the sample protein concentration and the Beads binding capacity, ~50µg protein/mg Beads.)
- 4. Mix the sample with beads for 1-2 minutes by slowly pipetting up and down 20-25 times *or* Vortex the sample for 5 minutes at 2000 rpm for the PCR tube or 800 rpm for Elisa plates.

## **IMPORTANT!**

• Users need to optimize the beads and free detergents ratio based on the binding capacity listed in table 1 and the Speed and time if using a vortex mixer.

2



# **Magnetic Beads Make Things Simple**

5. Place the sample plate or tube on the magnetic separator for 30 seconds or until the solution is clear.

(Option: centrifuge at 3500 rpm for 45 seconds)

6. 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
Deproteinization efficiency is low.	Too many proteins in your sample	•	Repeat the same procedure.
	• Check the sample pH and make sure the pH is ~ 4.5.	•	Adjust sample pH to 4.5.

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		

3