Immobilized TCEP Disulfide Reducing Kit

Introduction

BcMag™ Immobilized TCEP Disulfide Reducing Kit (TCEP reducing magnetic resins) uses specifically designed magnetic beads to efficiently reduce disulfide bonds in proteins, peptides, and other disulfide bond-containing molecules (Fig.1). TCEP ([tris(2-carboxyethyl) phosphine] reducing magnetic resins avoid the need for time-consuming and resin, spin column and gravity-flow column procedures to separate the reduced sample from the reducing agent.

Fig.1 Immobilized TCEP Disulfide Reducing Magnetic Beads

Immobilized TCEP Disulfide Reducing Beads allow for sample reduction and quick recovery of reducing agent-free samples. TCEP is an effective disulfide bond reducer in proteins, peptides, and other disulfide bond-containing molecules and is relatively unreactive to other functional groups. The trialkylphosphine TCEP is stable in aqueous solutions and does not oxidize as quickly as other reducing agents such as dithiotreitol (DTT) and -mercaptoethanol (BME). TCEP has little effect on common sulfhydryl-reactive chemicals (e.g., maleimide crosslinkers). Nonetheless, many protocols demand that the reduced sample be recovered separately from the reducing agent.

Magnetic resins have significant advantages over traditional chromatography, such as column, agarose, or non-magnetic resin. The magnetic bead-based format enables rapid high-yield processing of 96 samples within a short time, achieving recoveries of more than 95% for various samples. When using column-based technologies, processing multiple samples in academic research labs may necessitate a significant quantity of hand pipetting. This pipetting can discourage differences in the yield of target biomolecules between experiments and people. Staff and students may require extensive training and practice to produce constant protein yields. It is due to the numerous benefits of magnetic resins, such as their ease of use, rapid experimental protocols, suitability, and convenience for high throughput automated and miniaturized processing.

Feature and benefits:

- Excellent recovery free of reducing agent—Removing the reducing agent and recovering the reduced molecule without sample loss is a difficulty inherent in DTT or -mercaptoethanol (BME). Immobilized TCEP enables you to recover protein/peptides in a high yield (90% or higher) without dialyzing or desalting.
- Odorless—Unlike DTT or BME, Immobilized TCEP is odorless, allowing reductions to be performed on the bench top.
- Air stability inherent TCEP stability eliminates the need for particular measures to avoid oxidation when handling, using, or storing Immobilized TCEP Disulfide Reducing Beads.
- Simple to use—The immobilized TCEP reductant resin allows you to dispense the quantity of support needed for each application. Reductions can be carried out at various pH levels (from 4 to 9) and temperatures (5 to 95°C).

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Specification	
Composition	Magnetic Bead grafted with TCEP group on the surface.
Magnetization	~40 EMU/g
Type of Magnetization	Superparamagnetic
Effective Density	2.0 g/ml
Formulation	Lyophilized Powder
Loading	TCEP concentration > 12 mM /ml
Storage	Store at 4°C from light upon receipt

Products	Cat.No. BH101	Cat.No. BH102
BcMag™ Immobilized TCEP Disulfide Reducing Kit	100 mg (2ml)	500 mg (10 ml)

PROTOCOL

Notes:

- Reduction occurs over a wide pH (pH 4.0-9.0) and temperature range (5°-95°C).
- Most proteins can be effectively reduced without the use of a denaturant. However, adding a denaturant such as 6M guanidine will help expose interior disulfides to the immobilized TCEP and assure complete reduction. Urea is not suggested as a denaturant because it generates cyanates that react with sulfhydryl groups.
- Including 5-20 mM EDTA in the sample buffer during reduction prevents divalent ions such as Zn 2+, Cu 2+, and Mg 2+ from reoxidizing the sulfhydryl groups.
- Because disulfides regenerate over time, the reduced sample should be used promptly after reduction.
- TCEP-immobilized beads are intended for one-time usage only.

  The following protocol is an example of Disulfide Reduction**.** To get the best results, we recommend performing a titration to optimize the amount of beads used for each application. The protocol can be scaled up/down.

Buffer:

- 20 mM EDTA
- dH_2O (Milli-Q ultrapure water)

Equipment

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	

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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 of Disulfide Reduction**.** To get the best results, we recommend performing a titration to optimize the amount of beads used for each application. The protocol can be scaled up/down.

A. Magnetic Particles Preparation

1. Suspend the Bead with dH_2O at a concentration of 50 mg/ml.
2. Shake or vortex the bottle to completely resuspend the magnetic beads before using.

   Note: Do not allow the magnetic beads to sit for more than two minutes before dispensing.

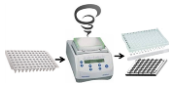
B. Sample preparation

1. Transfer desired amount of magnetic particles to a centrifuge tube.

   Note: Optimize the amount of beads used for each application. Typically, 30-50µl (3mg-5mg beads) of TCEP disulfide reducing magnetic beads for a 30-50 µL protein/peptide sample (50-80 µg).
2. Place the tube on the magnetic rack for 1-3 minutes until the supernatant becomes clear. Remove the supernatant while the tube remains on the rack.
3. Add the samples and completely mix by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2000 rpm.
4. Leave them at room temperature for 1 hour with end-over-end rotation.
5. Place the tube on a magnetic rack for 1-3 minutes. Transfer the supernatant to a fresh tube while the tube remains on the rack.

Mini-scale high throughput purification (30-50 µl)

1. Transfer 30-50µl beads to a new well of 96well PCR plate or 96-well microplates or 0.2ml PCR tube.
2. Place the tube on the magnetic rack for 1-3 minutes until the supernatant becomes clear. Remove the supernatant while the tube remains on the rack.
3. Add the the30-50µl samples and completely mix by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2500 rpm.



4. Leave them at room temperature for 1 hour with end-over-end rotation.
5. Place the sample plate/ tube on the magnetic separation plate for 30 seconds or until the solution is clear.
6. Transfer the supernatant to a fresh tube while the tube remains on the rack.

Troubleshooting

Problems	Possible Causes	Suggestion
Poor reduction of sample	Less amount of beads used	Use the recommended amount of beads
	Incubation time is too short.	Increase incubation time
	Disulfides were sterically inaccessible in protein.	Add 6 M guanidine•HCl to the reduction buffer.

	Incubation time is too long.	Do not exceed a 2-hour incubation
Loss of reducing the capacity of beads	The product was stored for more than one-year-old.	Purchase new product

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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