One-Step Dye Removal Kit

Introduction

Fluorescent dyes, also called reactive dyes or fluorophores, are natural or synthetic compounds that absorb light and re-emit it at a longer wavelength. Due to their unique advantages such as versatility, sensitivity, and quantitative capabilities**,** fluorescent dyes are widely used to label biologically relevant molecules such as protein, antibodies, peptides, DNA, and RNA for their applications in cell biology, immunology, biochemistry, microbiology, molecular biology, genomics, and proteomics. After a fluorescent labeling reaction, removing excess or unreacted dye from final conjugates is often necessary since it interferes with many downstream applications.

Removing fluorescent dyes is usually accomplished by spin columns, gel filtration, gravity-flow columns, and dialysis. However, those traditional methods present many problems, including time-consuming and labor-intensive processes, poor recovery of protein, peptides, or nucleic acids, and the challenge of adapting to automation. For this reason, we introduce a novel one-minute dye removal system.

BcMag™ One-Step Dye Removal Kit uses specially formulated resin with a proprietary surface chemistry to specifically remove the excess free (non-conjugated) fluorescent dyes from the finished protein, peptide or antibody labeling reaction. Compared with the dye removal columns, the resin can quickly and efficiently remove free dyes from the sample with just a single step and enables an individual or 96 samples to be processed simultaneously in less than 1 or 10 minutes with very little hands-on time (Fig.1). Since the magnetic resin only adsorbs the free dye, the labeled biomolecule recovery rate is exceptionally higher than >90%. Moreover, the magnetic beads can remove most of the dyes if the appropriate amount of samples and buffer conditions are used.

Workflow

The one-minute dye removal protocol is straightforward (Fig.1). 1. Add the beads directly to the sample. 2. Pipette or vortex to capture the free dye. 3. Magnetic separation of the beads from the protein solution, while the supernatant contains the purified and ready-to-run products

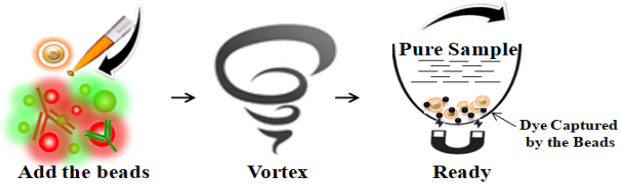

Fig.1

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 dye.
- Cost-effective: Eliminates columns, filters, and laborious repeat pipetting.
- High throughput: Compatible with many different automated liquid handling systems

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	Lyophilized Powder
Storage	Ship at room temperature, Store at 4° upon receipt.

PROTOCOL

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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	
Addition items are required if using 96-well PCR plates/tubes	
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 has to be ≥2.5mm.	
Addition items are required if using 96-well microplates	
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, it is necessary to optimize the time and speed, 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, as the examples listed in Table 1. Some dyes may require double processing because they require high molar excesses for labeling*.**

Table 1. Dye binding capacity

Fluorescent dyes	Binding capacity ng /mg beads**	Fluorescent dyes	Binding capacity ng /mg beads**
Alexa Fluor 546 C5-Maleimide	99.7	Alexa Fluor™ 514 NHS Ester	45.2
Cyanine 3 carboxylic acid	99.1	Cyanine 5 carboxylic acid	49.7
Cyanine 3 amine	99.3	Cyanine 3.5 carboxylic acid	99
Cyanine 5.5 amine	99.8	Cyanine 5.5 carboxylic acid	99.7
Cyanine 5 amine	49.85	Sulfo-Cyanine 5.5 amine	99.9
Sulfo-Cyanine3 amine	93.3	Sulfo-Cyanine5 carboxylic acid	24.9
DyLight™ 488 NHS Ester	90.5	DyLight™ 633 NHS Ester	87.4
Dylight 680-4x PEG NHS Ester	99.8	DyLight™ 405 NHS Ester	99
Oregon Green™ 488 carboxylic acid	84.2	FAM amine, 5-isomer	24.57
Rhodamine 5B amine	99.2	Texas Red™ hydrazide	890
Cibarcron blue F3GA	99.7	Fluorescein isothiocyanate	120.3
Bromocresol purple	105.2	Phenol red	99.5
Denim red	101	Bromophenol blue	99
Denim blue	104.2	SYBR® dye	102.4

****Assay condition: Mix 10 µl magnetic beads (100 mg/ml) with 100 µl protein sample (1:400 dilution of Human serum) containing different dyes at a concentration of 50 ng-800 ng in 0.1M Sodium phosphate, 0.15M NaCl, pH7.5 buffer, and vortex at 2000 rpm for 5 minutes. The dye removal efficiency is >93%, while protein recovery is >95%.**

1. Adjust the sample pH to 6.5 to 9.0 and the NaCl concentration to 150 mM.
2. 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.**
3. Add 10µl magnetic beads to a 100 µl sample containing free dyes.

   IMPORTANT!

   Users need to optimize the ratio of beads and free dyes since the free dye concentration varies from sample to sample due to the dye labeling efficiency.
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 (PCR plate or tube) or 800 rpm (96-well microplate).

   ***IMPORTANT!* Users need to optimize time and speed if using a vortex mixer.**
5. Place the sample plate or tube on the magnetic separation plate for 30 seconds or until the solution is clear.
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.

Troubleshooting

Problem	Probable cause	Suggestion
Low Protein Recovery	Vortexing time is too long.	• If using other digital vortex mixers, the vortex conditions such as speed and time must be optimized.
	Using too many magnetic beads	Thoroughly resuspend the magnetic beads and reduce the amounts of the beads.
Failure to remove dye.	Used inappropriate tubes or plates	Make sure 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 free dye in the sample	• Increasing either the speed or time • If using other digital vortex mixers, the vortex conditions 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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