One-Step DNA Fluorescent Labeling Cleanup 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, versatility, sensitivity, and quantitative capabilities**,** fluorescent dyes are widely used to label DNA/RNA as probes. End-labeling, nick-translation, and random primer production can all be used to label DNA/RNA molecules. The labeled DNA/RNA probes are widely used in molecular biology procedures such as gene library screening, identifying nucleotide sequences with blotting methods, and gene technologies such as nucleic acid and tissue microarrays. They can also be used to purify interacting molecules such as DNA binding proteins. DNA probes can be employed in environmental or health research to detect specific genes and bacteria in ambient or pathological materials via in-situ hybridization. After a fluorescent labeling reaction, removing excess or unreacted fluorescent dyes from the final labeling solution 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-step dye removal system.

BcMag™ One-Step DNA Fluorescent Labeling Cleanup Kit has specially formulated resin with proprietary surface chemistry. It removes the excess free (non-conjugated) fluorescent dyes, primer, dimer, adapter, salt, detergent, labeled dNTPs, dNTPs, and enzymes from the finished labeling reaction. The protocol is not only straightforward but also very flexible in removing different size DNA fragments by adjusting processing time, buffer pH, and detergent concentration (table1). 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 sample to be processed simultaneously in less than 1 or 10 minutes with very little hands-on time. Since the magnetic resin only adsorbs the free dye, primer, dimer, adapter, salt, detergent, dNTPs, and enzyme, the labeled DNA/RNA 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 (Table1).

Table 1

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

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.

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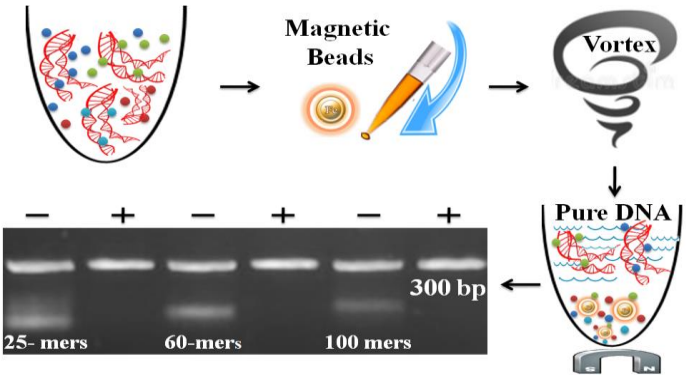

Fig.1 workflow of One-step Fluorescent Labeling Cleanup Kit

Features and Advantages:

- Simple protocol: No liquid transfer, One-tube, One-step
- Ultrafast: One-minute protocol
- Higher purity and recovery > 90% DNA.
- Effective Cleanup: Removes excess primer (<100- mer ssDNA), dimer, adapter, a salt such as Mg^{2+}, detergent, dNTPs, enzymes, and dye.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and ethanol
- High throughput: Compatible with many different automated liquid handling systems

Products

Components	Storage	50 preps Cat #: AK-101	100 preps Cat #: AK-102
BcMag™ One-Step Fluorescent Labeling Cleanup Kit	4°C	250 µL	500 µL

PROTOCOL

A. Materials Required by the User

- 18.2 MΩ.cm, DNase/RNase-Free Ultrapure Water
- Triton™ X-100, Sigma, Catalog # T8787
- Others

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• Bcg Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • Bcg Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • Bcg Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • Bcg 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)
Bcg 96-well Plate Magnetic Rack.	• Bcg 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

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1.5/2.0 mL centrifuge tube
96-well PCR Plates or 8-Strip PCR Tubes
PCR plates/tubes ** ***IMPORTANT!*** 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.

B. Procedure

Important!

1. The following protocol is optimized for the efficient cleanup of 10µl DNA sample. The protocol can be scaled up or down as needed. However, the procedure may need to be optimized if an alternative reaction scale is used.
2. Shake or vortex the bottle to completely resuspend the magnetic beads before using.
3. Do not allow the magnetic beads to sit for more than two minutes before dispensing.
4. Dilute organic solvent to 0.2-0.5% (final) with ultrapure water if the labeling reaction contains more than 0.5% organic solvent such as DMSO (Dimethyl sulfoxide) in the labeling solution.
5. Based on applications, the user should choose buffer conditions based on table1. For example, if the sample does not contain detergent, add 1 µL of 1% Triton™ X-100 solution to a 10 µL sample (final concentration is 0.1%).
6. Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green.

Table 1

DNA Fragment Removal						
Buffer / DNA	+ 0.1% Triton x-100, pH7.5	- 0.1% Triton x-100 pH7.5	+ 0.1% Triton x-100 pH 8.0	- 0.1% Triton x-100 pH 8.0	+ 0.1% Triton x-100 pH 8.8	- 0.1% Triton x-100 pH 8.8
dsDNA (100 bp)	No removal	removal	removal	removal	No removal	removal
dsDNA (150 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (200 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (300 bp)	No removal	No removal	No removal	No removal	No removal	No removal
ssDNA 100 mer	removal	removal	removal	removal	removal	removal
dsDNA- Double-Stranded DNA; ssDNA- Single-stranded DNA The assay was done by using the following conditions: 1. 10 mM Tris-HCl with or without 0.1% triton (final concentration) and three different: pH 7.5, pH 8.0 and pH 8.8						

1. Add 5 µL magnetic beads to the 10 µL DNA sample.

2. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.

3. Mix thoroughly for 1 minute by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2500 rpm.

4. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.

5. Place the sample plate on the magnetic separation plate for 30 seconds or until the solution is clear to separate beads from the solution.

6. Transfer the supernatant to a clean plate while the sample plate remains on the magnetic separation plate for downstream applications.

C. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA Recovery	Vortexing speed is too fast. Vortexing time is too long.	• Reducing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, must be optimized.
	Using too many magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.

Failure to remove impurities.	Used inappropriate PCR tubes or PCR plates	Make sure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates is ≥2.5mm.
	Vortex speed is too slow, or vortex time needs to be longer.	• Increasing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, has to be optimized.
	Using fewer magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.
	Strong secondary structure of DNA fragments (<50bp dsDNA or < 100 mer ssDNA)	Denature the sample by heating it at 95°C for 2 min.
	Too much primer, dimer, adaptor, free dye, and detergent	• Use more magnetic beads based on table 1 for dye binding capacity. • Perform the second round of purification by following the same protocol.

Related Product

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit, Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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