Alexa Fluor® Oligonucleotide Amine Labeling Kits

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Alexa Fluor® dye succinimidyl ester or TFP ester (Component A)	3 vials	NA	• ≤–20°C • Desiccate • Protect from light	When stored properly, the kit components are stable for at least 6 months.
Dimethylsulfoxide (DMSO) (Component B)	200 µL	NA		
Labeling buffer (Component C)	100 µL	0.1 M sodium borate, pH 8.5		
Number of Labelings: Each kit contains sufficient reagents for three labeling reactions (50 µg of oligonucleotide per reaction).				
Approximate Fluorescence Excitation and Emission, in nm: 490/520 nm for Alexa Fluor® 488; 650/670 nm for Alexa Fluor® 647.				

Introduction

Molecular Probes Oligonucleotide Amine Labeling Kits provide a simple method for labeling amine-modified oligonucleotides with Alexa Fluor® dyes. The Alexa Fluor® series of dyes represent the state of the art in synthesized organic dyes, showing very bright fluorescence and exceptional photostability. The dyes have pH-independent fluorescence and high water solubility, making them ideal for experiments performed in aqueous environments. Oligonucleotides labeled with Alexa Fluor® dyes can be used as primers and as probes in hybridization experiments. Note that because the Alexa Fluor® dyes in the kits are mixed isomers, labeled oligonucleotides may show two distinct mobilities on high-resolution electrophoretic gels, and should therefore not be used for automated DNA sequencing or similar gel-based assays.

Before You Begin

Materials Required but Not Provided

- Amine-labeled oligonucleotide, available from commercial suppliers of custom oligonucleotides.

Kit Optimization

The kits have been optimized for labeling 50 µg of a 5′-amine–modified oligonucleotide, 18 to 24 bases in length. Slightly shorter or longer oligonucleotides may be labeled by the same procedure; however, adjustments to the protocol may be necessary for greatly shorter or longer oligonucleotides. The reaction may be scaled up or down as long as the concentration of each component is not changed. The procedure has not been tested with oligonucleotides containing more than one amine. Following the labeling reaction, the conjugate may be purified from the reaction mixture by preparative gel electrophoresis or reverse-phase HPLC.

Preparing the Amine-Modified Oligonucleotide

To ensure that the oligonucleotide is free of interfering compounds, especially amines, such as triethylamine or Tris, and ammonium salts, we strongly recommend extracting and precipitating the sample prior to initiating the labeling reaction. We suggest the following protocol for 0.1–1 mg oligonucleotide (3–30 A_{260} units).

- Dissolve the oligonucleotide in 100 µL of dH_2O and extract three times with an equal volume of chloroform.
- Precipitate the oligonucleotide by adding one-tenth volume (10 µL) of 3 M NaCl and two and a half volumes (250 µL) of cold absolute ethanol. Mix well and place at –20°C for 30 minutes.
- Centrifuge the solution in a microcentrifuge at ~12,000 g for 30 minutes.
- Carefully remove the supernatant, rinse the pellet once or twice with cold 70% ethanol, and dry under a vacuum.
- Dissolve the dry pellet in dH_2O to achieve a final concentration of 25 µg/µL (4.2 mM for an 18-mer). This amine-modified oligonucleotide stock solution may be stored frozen at ≤–20°C.

Experimental Protocol

Performing the Reaction

1.1 Thaw the reagents. Remove one vial of reactive dye (Component A), the vial of DMSO (Component B), and one vial of labeling buffer (Component C) from the freezer and allow them to warm to room temperature completely before opening. Do not heat the buffer or reactive dye.

1.2 Dissolve the dye in solvent. Add 7 µL of DMSO (Component B) to the vial of reactive dye. Dissolve the material by pipetting up and down, washing the sides of the vial. For better visibility, you may want to peel off the paper label. The reactive dye should be freshly prepared for each labeling reaction. DO NOT STORE THE DYE FOR FUTURE USE.

1.3 Prepare the reaction mixture. To the vial containing the reactive label in DMSO, add:

- 41 µL labeling buffer (Component C)
- 2 µL of a 25 µg/µL oligonucleotide stock solution (preparation is described in the previous section, *Preparing the Amine-Modified Oligonucleotide*).

The reaction mixture may have a grainy appearance, but this will not adversely affect the conjugation. We strongly advise against attempting to improve the solubility of the label, because modifying the composition of the mixture can drastically reduce the labeling efficiency.

1.4 Incubate the reaction. Place the vial on a shaker oscillating at low speed for six hours (or overnight if more convenient) at room temperature. Gently vortex mix or tap the vial every half hour for the first two hours to ensure that the reaction remains well mixed. Do not mix violently, as material may be left on the sides of the vial. After six hours, 50–90% of the amine-modified oligonucleotide molecules should be labeled. The reaction may be incubated overnight, although this generally will not result in a greater labeling efficiency.

Purifying the Labeled Oligonucleotide

Following the reaction, the labeling mixture contains labeled oligonucleotide, unlabeled oligonucleotide, and unincorporated dye. The labeled oligonucleotide can be purified from the reaction mixture by preparative gel electrophoresis or reverse-phase HPLC. Regardless of the purification method selected, ethanol precipitation is recommended as the first step.

Ethanol precipitation of labeled oligonucleotide

Precipitate the reaction mixture with ethanol as follows: Add one-tenth volume of 3 M NaCl and two and a half volumes of cold absolute ethanol to the reaction vial. Mix well and place at –20°C for 30 minutes. Centrifuge the solution in a microcentrifuge at ~12,000 × g for a full 30 minutes. Loss of sample may occur if the centrifugation is not long enough. Carefully remove the supernatant, rinse the pellet once or twice with cold 70% ethanol and dry briefly. If the labeled oligonucelotide becomes completely dry, it will be difficult to redissolve.

- Some unreacted labeling reagent may have precipitated over the course of the reaction or may be stuck on the walls of the reaction vial. This material should be *completely* redissolved by extensive vortex mixing before centrifugation. Redissolving the labeling reagent ensures that the precipitated oligonucleotide will be minimally contaminated with unreacted label.
- In some cases, the labeled oligonucleotide may have already precipitated onto the walls of the reaction tube. This precipitate will not dissolve with the addition of NaCl and ethanol—the precipitated product will remain on the walls of the tube, however the free dye will dissolve and be eliminated. After centrifugation and rinsing, the pellet should be soluble.

Purification by HPLC

Labeled oligonucleotides can be purified by reverse-phase HPLC using a standard analytical (4.6 × 250 mm) C8 or C18 column. Dissolve the pellet from the ethanol precipitation in 0.1 M triethylammonium acetate (TEAA). Load the dissolved pellet onto the column in 0.1 M TEAA and run a linear 5–95% acetonitrile gradient over 30 minutes.

- There will be peaks that correspond to the unlabeled oligonucleotide, the labeled oligonucleotide, and the free dye. The actual order and number of these peaks may vary, depending on the length of the oligonucleotide and the purity of the sample. Note that Alexa Fluor® dyes contain multiple isomers, which may run slightly differently on the HPLC. We strongly recommend running a small portion of the sample on the column first, to ascertain the identity of the peaks before running preparative HPLC.
- To determine the identity of the peaks, monitor the absorbance at both 260 nm and at the absorbance maxima (λ_{max}) for the dye (see Table 1). For instruments with only one detector, two small samples should be run, each monitored at a different wavelength. Unlabeled oligonucleotide will show an absorbance at 260 nm only. Both the free dye and the labeled oligonucleotide will have absorbance at both 260 nm (A_{260}) and at the absorbance maximum of the dye (A_{max}); however, the labeled oligonucleotide will have a higher A_{260}:A_{max} ratio.
- For more details, please refer to Oliver R.W.A., *HPLC of Macromolecules: A Practical Approach*, IRL Press (1989).

Purification by gel electrophoresis

To purify the labeled oligonucleotide by gel electrophoresis, pour a 0.5 mm–thick polyacrylamide slab gel. For oligonucleotides less than 25 bases in length, use 19% acrylamide, for oligonucleotides 25–40 bases, 15% acrylamide, and for oligonucleotides 40–100 bases, 12% acrylamide. Resuspend the pellet from ethanol precipitation in 200 µL of 50% formamide, and incubate at 55°C for 5 minutes to disrupt any secondary structure. Load the warmed oligonucleotide onto the gel (you may need to use several wells) and load an adjacent well with 50% formamide plus 0.05% bromophenol blue. The bromophenol blue will migrate at approximately the same rate as the oligonucleotide. Run the gel until the bromophenol blue indicator dye is two-thirds of the way down the gel. Remove the gel from the glass plates and place on Saran Wrap. Lay the gel on a fluorescent TLC plate. Locate the labeled and unlabeled oligonucleotides by illumination with a handheld UV source. The Alexa Fluor® 488

fluorophore-labeled oligonucleotides will show a visible fluorescence when illuminated with UV light, although this may be somewhat quenched by the presence of bromophenol blue. The Alexa Fluor® 647 fluorophore-labeled oligonucleotides will not show visible fluorescence. Cut out the band containing the labeled oligonucleotide and elute by the "crush-and-soak" method or other suitable method. For more details, please refer to Sambrook J., Fritsch E.F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory (1989).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
A20191	Alexa Fluor® 488 Oligonucleotide Amine Labeling Kit *3 labelings*	1 kit
A20196	Alexa Fluor® 647 Oligonucleotide Amine Labeling Kit *3 labelings*	1 kit

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