

## Modification of Peptides, Oligonucleotides and Other Small Biomolecules with Tide Fluor™ (TF) Dye Succinimidyl Esters

### Introduction

The sulfonated cyanine dyes have improved fluorescence quantum yield than those of non-sulfonate cyanines. They are commercially available as Cy3®, Cy5®, Cy5.5® and Cy7®. However, these commercial protein-labeling Cy Dyes and Alexa Fluor® dyes are cost-prohibitive for labeling peptides and oligonucleotides that require much larger amount of labeling dyes. The improved and affordable Tide Fluor™ dyes are optimized as building blocks for developing FRET oligonucleotides and peptides. Our Tide Fluor™ dyes have stronger fluorescence and higher photostability than the classic fluorophores (such as fluoresceins, rhodamines and cyanines). They are the best affordable fluorescent dyes for labeling peptides and oligonucleotides without comprised performance.

- **Optimized to label peptides and nucleotides with performance comparable to Alexa Fluor® Dyes**
- **Optimized to pair with Tide Quencher™ dark acceptors to maximize FRET efficiency**
- **pH-insensitive and environment-insensitive fluorescence for developing robust assays**
- **Higher photostability to improve the quality of fluorescence imaging**
- **A variety of reactive forms available for conjugations**

### Labeling Mechanism



Succinimidyl esters are proven to be the best reagents for amine modifications because the amide bonds that are formed are essentially identical to, and as stable as the natural peptide bonds. These reagents are generally stable and show good reactivity and selectivity with aliphatic amines. There are few factors that need be considered when SE compounds are used for conjugation reaction:

- 1). *Solvents*: For the most part, reactive dyes are hydrophobic molecules and should be dissolved in anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO).
- 2). *Reaction pH*: The labeling reactions of amines with succinimidyl esters are strongly pH dependent. Amine-reactive reagents react with non-protonated aliphatic amine groups, including the terminal amines of proteins and the  $\epsilon$ -amino groups of lysines. Thus amine acylation reactions are usually carried out above pH 7.5. Protein modifications by succinimidyl esters can typically be done at pH 7.5-8.5, whereas isothiocyanates may require a pH 9.0-10.0 for optimal conjugations.
- 3). *Reaction Buffers*: Buffers that contain free amines such as Tris and glycine and thiol compounds must be avoided when using an amine-reactive reagent. Ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation must also be removed (such as viadilysis) before performing dye conjugations.
- 4). *Reaction Temperature*: Most conjugations are done at room temperature. However, either elevated or reduced temperature may be required for a particular labeling reaction.

### Storage and Handling

The dye labeled oligos should be stored at  $\leq -15^{\circ}\text{C}$ , and kept from light. For longer storage, dye labeled oligo could be divided as single-used aliquot and stored at  $\leq -15^{\circ}\text{C}$ . PROTECT FROM LIGHT.

### **Sample Protocols (FOR REFERENCE PURPOSE ONLY)**

Always wear the protection apparatus (such as gloves). Warm all the components before opening them, and immediately prepare the required solutions before starting the conjugation. Avoid repeated freezing and thawing if possible. Any solutions containing the dye should be kept from light.

### **Label Amino-Modified Oligonucleotides with Tide Fluor™ Dyes**

The following protocol has been optimized for labeling 200 µg (~6 A<sub>260 nm</sub> units) of a proprietary oligonucleotide. You need modify the protocol to get the best results for your particular application by multiple experimentations. **YOUR AMINO-MODIFIED OLIGO MUST BE TREATED TO REMOVE AMMONIA THAT RAPIDLY REACTS AND CONSUMES DYE SUCCINIMIDYL ESTERS.**

#### **1. Prepare Oligo Solution (Solution A)**

Dissolve your amino-modified oligo (~200 µg) in a tetraborate buffer (100 µL, pH 8.5±0.5).

*Note 1:* The oligonucleotide must be synthesized with an amine group. See Appendix 1 for the purification of amino-modified oligos.

*Note 2:* Avoid buffers that contain primary amines, such as Tris, as these compete for conjugation with the amine-reactive compound.

#### **2. Prepare Dye Solution (Solution B)**

Dissolve 1 mg dye SE in 100 µL DMSO (>10 mg/mL if possible) by pipetting up and down. Centrifuge the solution stock on the sides of the vial to the vial bottom.

*Note:* prepare the DMSO dye solution before starting the conjugation. Extended storage of the dye solution may reduce the dye activity. Any solutions containing the dye should be kept from light. We do not recommend that you store the DMSO dye solution for future use.

#### **3. Run Conjugation Reaction**

3.1 To the dye solution (B, 20-50 µL) add the oligo solution (A, 100 µL) with stirring or shaking (keeping the reaction mixture from light).

3.2 Rotate or shake the reaction mixture for 4-6 hours at room temperature on a rotator or shaker.

*Note:* Gently vortex tap the vial every 10 minutes for the first hour to ensure that the reaction solution 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 might be incubated overnight if it is more convenient. However, overnight incubation will not result in a greater labeling efficiency in most cases.

#### **4. Purify Dye-Oligo Conjugate**

4.1 Preliminary purification by ethanol precipitation of labeled oligonucleotide

a. Add 20 µL (one-tenth reaction solution volume in general) of 3 M NaCl and 300 µL cold absolute ethanol (two and half reaction solution volume in general) to the reaction vial.

b. Mix the solution well and place it at –20°C for 30 minutes.

c. Centrifuge the solution in a microcentrifuge at 10,000 to 15,000 × g for 30 minutes.

*Note:* Loss of sample may occur if the centrifugation is not long enough.

d. Carefully remove the supernatant, rinse the pellet 1-3 times with cold 70% ethanol and dry briefly.

*Note:* 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.

4.2 Final Purification by HPLC or by gel electrophoresis

See Appendix I

### Label Peptides with Tide Fluor™ Dyes

The following protocol has been optimized for labeling 10 mg of a proprietary peptide (MW ~ 2000) that contains only a single free amino group. **YOU NEED MODIFY THE PROTOCOL TO ARCHIE THE BEST RESULTS FOR YOUR PARTICULAR APPLICATION BY MULTIPLE EXPERIMENTATIONS.**

#### **1. Prepare Peptide Solution (Solution A)**

Dissolve your peptide (~10 mg) in DMF (~1 ml).

*Note 1:* The peptide must be neutralized with a base such as triethylamine or potassium carbonate.

*Note 2:* Avoid buffers that contain primary amines, such as Tris, as these compete for conjugation with the amine-reactive compound.

#### **2. Prepare Dye Solution (Solution B)**

Dissolve 5 mg dye SE in 500 µL DMF (>10 mg/mL if possible) by pipetting up and down.

*Note:* prepare the DMF dye solution before starting the conjugation. Extended storage of the dye solution may reduce the dye activity. Any solutions containing the dye should be kept from light. We do not recommend that you store the DMF dye solution for future use.

#### **3. Run Conjugation Reaction**

3.3 To the dye solution (B, 500 µL) add the peptide solution (A, 1 mL) with stirring or shaking (keeping the reaction mixture from light).

3.4 Stir the reaction mixture for 4-6 hours at room temperature.

#### **4. Purify Dye-Peptide Conjugate**

The reaction solution was concentrated and purified on a C18 column to afford the desired conjugate. The fractions were analyzed by HPLC, and the fractions of >97% purity were pooled and lyophilized.

*Note 1:* HPLC Purification Conditions: TEAB buffer (triethyl ammonium bicarbonate, 0.25 mmol, pH=7.0-8.0) was used as buffer A and acetonitrile as buffer B. The HPLC was run from 0% B to 30% B in 60 min (flow rate: 100 mL/min).

*Note 2:* Avoid strong light during the operation.

### References

1. Hermanson GT (1996). *Biocojugate Techniques*, Academic Press, New York.
2. Sambrook J., Fritsch E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor Laboratory.
3. Brinkley M (1992). A brief survey of methods for preparing protein conjugates with dyes, haptens, and cross-linking reagents. *Bioconjug Chem* **3**, 2-13.

## Appendix I. Chemical Properties of Tide Fluor™ Fluorescent Labeling Dyes

Tide Fluor™ dyes have improved labeling performance than the classic fluorescent labeling dyes such as FITC, TRITC, Texas Red®, Cy3, Cy5 and Cy7. They are the best affordable fluorescent dyes (alternative to Alexa Fluor® dyes) for labeling oligos and peptides without compromised performance. Each Tide Fluor™ dye is developed to match the spectral properties of a particular Alexa Fluor® or other labeling dyes (such as DyLight™ dyes).

| Labeling Dye | Cat# | Product Description                           | Reactivity             | Adduct MW Calculation* |
|--------------|------|---|------------------------|------------------------|
| TF1          | 2236 | Tide Fluor™ 1 azide [TF1 azide]               | Azide                  | + 301                  |
|              | 2237 | Tide Fluor™ 1 alkyne [TF1 alkyne]             | Alkyne                 | + 270                  |
|              | 2238 | Tide Fluor™ 1 acid [TF1 acid]                 | NH <sub>2</sub> and OH | + 215                  |
|              | 2239 | Tide Fluor™ 1 amine [TF1 amine]               | CO <sub>2</sub> H      | + 257                  |
|              | 2242 | Tide Fluor™ 1 maleimide [TF1 maleimide]       | SH                     | + 355                  |
|              | 2244 | Tide Fluor™ 1 succinimidyl ester [TF1 SE]     | Aliphatic amine        | + 215                  |
| TF2          | 2245 | Tide Fluor™ 2 acid [TF2 acid]                 | NH <sub>2</sub> and OH | + 469                  |
|              | 2246 | Tide Fluor™ 2 amine [TF2 amine]               | CO <sub>2</sub> H      | + 398                  |
|              | 2247 | Tide Fluor™ 2 maleimide [TF2 maleimide]       | SH                     | + 680                  |
|              | 2248 | Tide Fluor™ 2 succinimidyl ester [TF2 SE]     | Aliphatic amine        | + 469                  |
|              | 2252 | Tide Fluor™ 2 azide [TF2 azide]               | Azide                  | + 555                  |
|              | 2253 | Tide Fluor™ 2 alkyne [TF2 alkyne]             | Alkyne                 | + 524                  |
| TF2WS        | 2348 | Tide Fluor™ 2WS acid [TF2WS acid]             | NH <sub>2</sub> and OH | + 628                  |
|              | 2349 | Tide Fluor™ 2WS succinimidyl ester [TF2WS SE] | Aliphatic amine        | + 628                  |
| TF3          | 2254 | Tide Fluor™ 3 azide [TF3 azide]               | Azide                  | + 526                  |
|              | 2255 | Tide Fluor™ 3 alkyne [TF3 alkyne]             | Alkyne                 | + 495                  |
|              | 2268 | Tide Fluor™ 3 acid [TF3 acid]                 | NH <sub>2</sub> and OH | + 440                  |
|              | 2269 | Tide Fluor™ 3 amine [TF3 amine]               | CO <sub>2</sub> H      | + 496                  |
|              | 2270 | Tide Fluor™ 3 maleimide [TF3 maleimide]       | SH                     | + 580                  |
|              | 2271 | Tide Fluor™ 3 succinimidyl ester [TF3 SE]     | Aliphatic amine        | + 440                  |
| TF3WS        | 2345 | Tide Fluor™ 3WS acid [TF3WS acid]             | NH <sub>2</sub> and OH | + 706                  |
|              | 2346 | Tide Fluor™ 3WS succinimidyl ester [TF3WS SE] | Aliphatic amine        | + 706                  |
| TF4          | 2285 | Tide Fluor™ 4 acid [TF4 acid]                 | NH <sub>2</sub> and OH | + 544                  |
|              | 2286 | Tide Fluor™ 4 amine [TF4 amine]               | CO <sub>2</sub> H      | + 586                  |
|              | 2287 | Tide Fluor™ 4 maleimide [TF4 maleimide]       | SH                     | + 684                  |
|              | 2289 | Tide Fluor™ 4 succinimidyl ester [TF4 SE]     | Aliphatic amine        | + 544                  |
|              | 2300 | Tide Fluor™ 4 azide [TF4 azide]               | Azide                  | + 630                  |
|              | 2301 | Tide Fluor™ 4 alkyne [TF4 alkyne]             | Alkyne                 | + 599                  |
| TF5WS        | 2275 | Tide Fluor™ 5WS azide [TF5WS azide]           | Azide                  | + 1078                 |
|              | 2276 | Tide Fluor™ 5WS alkyne [TF5WS alkyne]         | Alkyne                 | + 787                  |
|              | 2278 | Tide Fluor™ 5WS acid [TF5WS acid]             | NH <sub>2</sub> and OH | + 732                  |
|              | 2279 | Tide Fluor™ 5WS amine [TF5WS amine]           | CO <sub>2</sub> H      | + 774                  |
|              | 2280 | Tide Fluor™ 5WS maleimide [TF5WS maleimide]   | SH                     | + 873                  |
|              | 2281 | Tide Fluor™ 5WS succinimidyl ester [TF5WS SE] | Aliphatic amine        | + 732                  |

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|              |      |   |                        |        |
|--------------|------|---|------------------------|--------|
| <b>TF6WS</b> | 2291 | Tide Fluor™ 6WS acid [TF6WS acid]             | NH <sub>2</sub> and OH | + 899  |
|              | 2292 | Tide Fluor™ 6WS amine [TF6WS amine]           | CO <sub>2</sub> H      | + 941  |
|              | 2293 | Tide Fluor™ 6WS maleimide [TF6WS maleimide]   | SH                     | + 1039 |
|              | 2294 | Tide Fluor™ 6WS succinimidyl ester [TF6WS SE] | Aliphatic amine        | + 899  |
|              | 2302 | Tide Fluor™ 6WS azide [TF6WS azide]           | Azide                  | + 1079 |
|              | 2303 | Tide Fluor™ 6WS alkyne [TF6WS alkyne]         | Alkyne                 | + 1048 |
| <b>TF7WS</b> | 2304 | Tide Fluor™ 7WS azide [TF7WS azide]           | Azide                  | + 845  |
|              | 2305 | Tide Fluor™ 7WS alkyne [TF7WS alkyne]         | Alkyne                 | + 813  |
|              | 2330 | Tide Fluor™ 7WS acid [TF7WS acid]             | NH <sub>2</sub> and OH | + 758  |
|              | 2331 | Tide Fluor™ 7WS amine [TF7WS amine]           | CO <sub>2</sub> H      | + 801  |
|              | 2332 | Tide Fluor™ 7WS maleimide [TF7WS maleimide]   | SH                     | + 899  |
|              | 2333 | Tide Fluor™ 7WS succinimidyl ester [TF7WS SE] | Aliphatic amine        | + 758  |
| <b>TF8WS</b> | 2306 | Tide Fluor™ 8WS azide [TF8WS azide]           | Azide                  | + 1011 |
|              | 2307 | Tide Fluor™ 8WS alkyne [TF8WS alkyne]         | Alkyne                 | + 980  |
|              | 2335 | Tide Fluor™ 8WS acid [TF8WS acid]             | NH <sub>2</sub> and OH | + 925  |
|              | 2336 | Tide Fluor™ 8WS amine [TF8WS amine]           | CO <sub>2</sub> H      | + 967  |
|              | 2337 | Tide Fluor™ 8WS maleimide [TF8WS maleimide]   | SH                     | + 1065 |
|              | 2338 | Tide Fluor™ 8WS succinimidyl ester [TF8WS SE] | Aliphatic amine        | + 925  |

\* The molecular weight of the desired conjugate = the molecular weight of the free unlabeled molecule + the value listed in the table.

## **Appendix II. Spectral Properties of Tide Fluor™ Fluorescent Labeling Dyes**

| <b>Labeling Dye</b> | <b>Extinction Coefficient<sup>1</sup> (cm<sup>-1</sup>M<sup>-1</sup>)</b> | <b>Abs (nm)</b> | <b>Em (nm)</b> | <b>FQY<sup>2</sup></b> | <b>CF at 260 nm<sup>3</sup></b> | <b>CF at 280 nm<sup>4</sup></b> |
|---------------------|---|-----------------|----------------|------------------------|---------------------------------|---------------------------------|
| <b>TF1</b>          | 20,000  | 345             | 442            | 0.95                   | 0.246                           | 0.187                           |
| <b>TF2</b>          | 75,000  | 500             | 527            | 0.90                   | 0.288                           | 0.201                           |
| <b>TF2WS</b>        | 75,000  | 502             | 525            | 0.90                   | 0.211                           | 0.091                           |
| <b>TF3</b>          | 85,000  | 555             | 584            | 0.85                   | 0.331                           | 0.201                           |
| <b>TF3WS</b>        | 150,000   | 555             | 565            | 0.10 <sup>5</sup>      | 0.079                           | 0.079                           |
| <b>TF4</b>          | 90,000  | 590             | 618            | 0.91                   | 0.489                           | 0.436                           |
| <b>TF5WS</b>        | 250,000   | 649             | 664            | 0.25                   | 0.023                           | 0.027                           |
| <b>TF6WS</b>        | 220,000   | 676             | 695            | 0.18                   | 0.111                           | 0.009                           |
| <b>TF7WS</b>        | 275,000   | 749             | 775            | 0.12                   | 0.009                           | 0.049                           |
| <b>TF8WS</b>        | 250,000   | 775             | 807            | 0.08                   | 0.103                           | 0.109                           |

Notes: 1. Extinction Coefficient at their maximum absorption wavelength; 2. FQY = fluorescence quantum yield in aqueous buffer (pH 7.2); 3. CF at 260 nm is the correction factor used for eliminating the dye contribution to the absorbance at 260 nm (for oligo and nucleic acid labeling); 3. CF at 280 nm is the correction factor used for eliminating the dye contribution to the absorbance at 280 nm (for peptide and protein labeling); 5. Fluorescence intensity is significantly increased upon coupled to proteins or long peptides.

### **Appendix III. FRET Selection Guide of Tide Quencher™ Dyes**

| <b>Tide Fluor™ Donor</b>                       | <b>Ex(nm)</b>    | <b>Em (nm)</b>   | <b>Features and Benefits</b>  | <b>Ordering Information</b>  |
|--|------------------|------------------|---|--|
| Tide Fluor™ 1 (TF1)                            | 345 nm           | 442 nm           | <i>Alternative to EDANS</i><br><ul style="list-style-type: none"> <li>• Much stronger absorption</li> <li>• Much stronger fluorescence</li> <li>• Less environment-sensitive</li> </ul> | #2236 & 2237 (TF1 Click chemistry)<br>#2238 (TF1 acid)<br>#2239 (TF1 amine)<br>#2242 (TF1 maleimide, SH-reactive)<br>#2244 (TF1 SE, NH <sub>2</sub> -reactive)             |
| Tide Fluor™ 2 (TF2)<br>Tide Fluor™ 2WS (TF2WS) | 500 nm<br>502 nm | 527 nm<br>525 nm | <i>Alternative to FAM, FITC and Alexa Fluor® 488</i><br>pH-insensitive fluorescence<br>Photostable  | #2245 (TF2 acid) & 2348 (TF2WS acid)<br>#2246 (TF2 amine)<br>#2247 (TF2 maleimide, SH-reactive)<br>#2248 (TF2, SE) & #2249 (TF2WS SE)<br>#2252 & 2253 (Click chemistry)    |
| Tide Fluor™ 3 (TF3)<br>Tide Fluor™ 3WS (TF3WS) | 555 nm<br>555 nm | 584 nm<br>565 nm | <i>Alternative to Cy3® and Alexa Fluor® 555</i><br>Strong fluorescence<br>Photostable   | #2254 & 2255 (TF3 Click chemistry)<br>#2268 (TF3 acid) & 2345 (TF3WS acid)<br>#2269 (TF3 amine)<br>#2270 (TF3 maleimide, SH-reactive)<br>#2271 (TF3 SE) & #2346 (TF3WS SE) |
| Tide Fluor™ 4 (TF4)                            | 590 nm           | 618 nm           | <i>Alternative to ROX, Texas Red® and Alexa Fluor® 594</i><br>Strong fluorescence<br>Photostable  | #2285 (TF4 acid)<br>#2286 (TF4 amine)<br>#2287 (TF4 maleimide, SH-reactive)<br>#2289 (TF4 SE, NH <sub>2</sub> -reactive)<br>#2300 & 2301 (TF4 Click chemistry)             |
| Tide Fluor™ 5WS (TF5WS)                        | 649 nm           | 664 nm           | <i>Alternative to Cy5® and Alexa Fluor® 647</i><br>Strong fluorescence<br>Photostable   | #2275 & 2276 (TF5WS Click chemistry)<br>#2278 (TF5WS acid)<br>#2279 (TF5WS amine)<br>#2280 (TF5WS maleimide, SH-reactive)<br>#2281 (TF5WS SE, NH <sub>2</sub> -reactive)   |
| Tide Fluor™ 6WS (TF6WS)                        | 676 nm           | 695 nm           | <i>Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680</i><br>Strong fluorescence<br>Photostable   | #2291 (TF6WS acid)<br>#2292 (TF6WS amine)<br>#2293 (TF6WS maleimide, SH-reactive)<br>#2294 (TF6WS SE, NH <sub>2</sub> -reactive)<br>#2302 & 2303 (TF6WS Click chemistry)   |
| Tide Fluor™ 7WS (TF7WS)                        | 749 nm           | 775 nm           | <i>Alternative to Cy7® and Alexa Fluor® 750</i><br>Strong fluorescence<br>Photostable   | #2304 & 2305 (TF7WS Click chemistry)<br>#2330 (TF7WS acid)<br>#2331 (TF7WS amine)<br>#2332 (TF7WS maleimide, SH-reactive)<br>#2333 (TF7WS SE, NH <sub>2</sub> -reactive)   |
| Tide Fluor™ 8WS (TF8WS)                        | 775 nm           | 807 nm           | <i>Alternative to IRDye® 800</i><br>Stronger fluorescence<br>Higher Photostability  | #2306 & 2307 (TF4 Click chemistry)<br>#2335 (TF8WS acid)<br>#2336 (TF8WS amine)<br>#2337 (TF8WS maleimide, SH-reactive)<br>#2338 (TF8WS SE, NH <sub>2</sub> -reactive)     |

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## **Appendix IV. HPLC Purification of Dye Oligonucleotide Conjugates**

### **Ethanol Precipitation**

Some commercial oligonucleotides often contain some interfering compounds, especially amines, such as triethylamine or Tris, and ammonium salts, we strongly recommend you to extract and precipitate the commercial oligo samples prior to initiating your labeling reaction. On the other hand, the labeling mixture contains labeled oligonucleotide, unlabeled oligonucleotide, hydrolyzed dye acid and unincorporated dye SE. The impurities of hydrolyzed dye acid and unincorporated dye SE resulted from the labeling reaction can be effectively removed by ethanol precipitation. The following protocol was optimized for the further purification of 0.1–1 mg commercial oligonucleotide sample that was purified by HPLC (3–30 A260 units).

- 1) Dissolve your target oligonucleotide in 100  $\mu\text{L}$  of deionized water and extract three times with an equal volume of chloroform.
- 2) Precipitate the oligonucleotide by adding one-tenth volume (10  $\mu\text{L}$ ) of 3 M NaCl and two and a half volumes (250  $\mu\text{L}$ ) of cold absolute ethanol. Mix well and place at  $-20^{\circ}\text{C}$  for 30 minutes.
- 3) Centrifuge the solution in a microcentrifuge at 10,000 to 15,000 g for 30 minutes.
- 4) Carefully remove the supernatant, rinse the pellet 1-3 times with cold 70% ethanol, and dry under a vacuum.
- 5) Dissolve the dry pellet in deionized water to achieve a final concentration of  $>50 \mu\text{g}/\mu\text{L}$ . This amine-modified oligonucleotide stock solution may be immediately used or stored frozen at  $\leq -15^{\circ}\text{C}$ .

### **Purification by HPLC**

Labeled oligonucleotides can be purified by reverse-phase HPLC using a standard analytical C8 or C18 column using an analytical or semi-preparative HPLC instrument. The following protocol was optimized for the further purification of 0.1–1 mg labeled oligonucleotide (3–30 A260 units).

- 1) Dissolve the pellet from the ethanol precipitation in 0.1 M triethylammonium acetate (TEAA).
- 2) Load the dissolved pellet onto the column in 0.1 M TEAA and run a linear 5–95% acetonitrile gradient over 30 minutes.  
*Note 1: 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 depends on the length of the oligonucleotide and the purity of the sample.*  
*Note 2: To determine the identity of the peaks, monitor the absorbance at both 260 nm and at the absorbance maxima ( $\lambda_{\text{max}}$ ) for the dye. 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 (A260 for oligo) and at the absorbance maximum of the dye (Amax for dye); The dye-labeled oligonucleotide will have a higher A260:Amax ratio than the dye or hydrolyzed dye.*

### **Purification by Gel Electrophoresis**

- 1) Pour a 0.5 mm-thick polyacrylamide slab gel.  
*Note: 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.*
- 2) Resuspend the pellet from ethanol precipitation in 200  $\mu\text{L}$  of 50% formamide, and incubate at  $55^{\circ}\text{C}$  for 5 minutes to disrupt any secondary structure.
- 3) Load the warmed oligonucleotide onto the gel 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.  
*Note: You may need to use several wells.*
- 4) Run the gel until the bromophenol blue indicator dye is two-thirds of the way down the gel.
- 5) Remove the gel from the glass plates and place on Saran Wrap.
- 6) Lay the gel on a fluorescent TLC plate.
- 7) Locate the labeled and unlabeled oligonucleotides by illumination with a handheld UV source.