

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 CyDyes 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 compromised 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 ϵ -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 (i.e., via dialysis) 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 $A_{260\text{ nm}}$ 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 on the 5' end. See Appendix IV 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 volumes 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 IV.

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). *Bioconjugate 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.

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 ₂ and OH	+ 215
	2239	Tide Fluor™ 1 amine [TF1 amine]	CO ₂ H	+ 257
	2242	Tide Fluor™ 1 maleimide [TF1 maleimide]	SH	+ 355
	2244	Tide Fluor™ 1 succinimidyl ester [TF1 SE]	Aliphatic amine	+ 215
	2240	Tide Fluor™ 1 CPG [TF1 CPG] *500 Å*	Oligo Synthesis	+ 288
	2241	Tide Fluor™ 1 CPG [TF1 CPG] *1000 Å*	Oligo Synthesis	+ 288
TF2	2245	Tide Fluor™ 2 acid [TF2 acid]	NH ₂ and OH	+ 469
	2246	Tide Fluor™ 2 amine [TF2 amine]	CO ₂ H	+ 511
	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 ₂ and OH	+ 628
	2351	Tide Fluor™ 2WS amine [TF2WS amine]	CO ₂ H	+ 558
	2350	Tide Fluor™ 2WS maleimide [TF2WS maleimide]	SH	+ 767
	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 ₂ and OH	+ 440
	2269	Tide Fluor™ 3 amine [TF3 amine]	CO ₂ H	+ 496
	2270	Tide Fluor™ 3 maleimide [TF3 maleimide]	SH	+ 651
	2274	Tide Fluor™ 3 phosphoramidite [TF3 CEP]	Oligo Synthesis	+ 633
	2271	Tide Fluor™ 3 succinimidyl ester [TF3 SE]	Aliphatic amine	+ 440
TF3WS	2345	Tide Fluor™ 3WS acid [TF3WS acid]	NH ₂ and OH	+ 706
	2347	Tide Fluor™ 3WS amine [TF3WS amine]	CO ₂ H	+ 748
	2344	Tide Fluor™ 3WS maleimide [TF3WS maleimide]	SH	+ 846
	2346	Tide Fluor™ 3WS succinimidyl ester [TF3WS SE]	Aliphatic amine	+ 706
TF4	2285	Tide Fluor™ 4 acid [TF4 acid]	NH ₂ and OH	+ 544
	2286	Tide Fluor™ 4 amine [TF4 amine]	CO ₂ H	+ 586
	2287	Tide Fluor™ 4 maleimide [TF4 maleimide]	SH	+ 755
	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

[Continued on next page]

TF5WS	2275	Tide Fluor™ 5WS azide [TF5WS azide]	Azide	+ 818
	2276	Tide Fluor™ 5WS alkyne [TF5WS alkyne]	Alkyne	+ 787
	2278	Tide Fluor™ 5WS acid [TF5WS acid]	NH ₂ and OH	+ 732
	2279	Tide Fluor™ 5WS amine [TF5WS amine]	CO ₂ H	+ 774
	2280	Tide Fluor™ 5WS maleimide [TF5WS maleimide]	SH	+ 872
	2281	Tide Fluor™ 5WS succinimidyl ester [TF5WS SE]	Aliphatic amine	+ 732
TF6WS	2291	Tide Fluor™ 6WS acid [TF6WS acid]	NH ₂ and OH	+ 898
	2292	Tide Fluor™ 6WS amine [TF6WS amine]	CO ₂ H	+ 941
	2293	Tide Fluor™ 6WS maleimide [TF6WS maleimide]	SH	+ 1039
	2294	Tide Fluor™ 6WS succinimidyl ester [TF6WS SE]	Aliphatic amine	+ 898
	2302	Tide Fluor™ 6WS azide [TF6WS azide]	Azide	+ 1079
	2303	Tide Fluor™ 6WS alkyne [TF6WS alkyne]	Alkyne	+ 1048
TF7WS	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 ₂ and OH	+ 758
	2331	Tide Fluor™ 7WS amine [TF7WS amine]	CO ₂ H	+ 801
	2332	Tide Fluor™ 7WS maleimide [TF7WS maleimide]	SH	+ 899
	2333	Tide Fluor™ 7WS succinimidyl ester [TF7WS SE]	Aliphatic amine	+ 758
TF8WS	2306	Tide Fluor™ 8WS azide [TF8WS azide]	Azide	+ 1010
	2307	Tide Fluor™ 8WS alkyne [TF8WS alkyne]	Alkyne	+ 979
	2335	Tide Fluor™ 8WS acid [TF8WS acid]	NH ₂ and OH	+ 925
	2336	Tide Fluor™ 8WS amine [TF8WS amine]	CO ₂ 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

Labeling Dye	Extinction Coefficient ¹ (cm ⁻¹ M ⁻¹)	Abs (nm)	Em (nm)	FQY ²	CF at 260 nm ³	CF at 280 nm ⁴
TF1	20,000	341	448	0.95	0.246	0.187
TF2	75,000	503	525	0.90	0.288	0.09
TF2WS	75,000	491	516	0.90	0.211	0.11
TF3	75,000	554	578	0.85	0.331	0.179
TF3WS	150,000	551	563	0.10 ⁵	0.079	0.079
TF4	90,000	578	602	0.91	0.489	0.436
TF5WS	250,000	649	664	0.25	0.023	0.027
TF6WS	220,000	682	701	0.18	0.111	0.101
TF7WS	275,000	756	780	0.12	0.009	0.049
TF8WS	250,000	785	801	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 Fluor™ Dyes

Tide Fluor™ Donor	Ex(nm)	Em (nm)	Features and Benefits	Ordering Information
Tide Fluor™ 1 (TF1)	341 nm	448 nm	<i>Alternative to EDANS</i> <ul style="list-style-type: none"> • Much stronger absorption • Much stronger fluorescence • Less environment-sensitive 	#2236 (TF1 azide, Click chemistry) #2237 (TF1 alkyne, Click chemistry) #2238 (TF1 acid) #2239 (TF1 amine) #2242 (TF1 maleimide, SH-reactive) #2193 & #2194 (TF1 CPG, OH-reactive) #2244 (TF1 SE, NH ₂ -reactive)
Tide Fluor™ 2 (TF2) Tide Fluor™ 2WS (TF2WS)	503 nm 491 nm	525 nm 516 nm	<i>Alternative to FAM, FITC and Alexa Fluor® 488</i> <ul style="list-style-type: none"> • pH-insensitive fluorescence • Photostable 	#2245 (TF2 acid) & 2348 (TF2WS acid) #2246 (TF2 amine) & 2351 (TF2WS amine) #2247 (TF2 maleimide, SH-reactive) #2350 (TF2WS maleimide, SH-reactive) #2248 (TF2, SE) & #2249 (TF2WS SE) #2252 (TF2 azide, Click chemistry) #2253 (TF2 alkyne, Click chemistry)
Tide Fluor™ 3 (TF3) Tide Fluor™ 3WS (TF3WS)	554 nm 551 nm	578 nm 563 nm	<i>Alternative to Cy3® and Alexa Fluor® 555</i> <ul style="list-style-type: none"> • Strong fluorescence • Photostable 	#2254 (TF3 azide, Click chemistry) #2255 (TF3 alkyne, Click chemistry) #2268 (TF3 acid) & 2345 (TF3WS acid) #2269 (TF3 amine) & 2347 (TF3WS amine) #2270 (TF3 maleimide, SH-reactive) #2344 (TF3WS maleimide, SH-reactive) #2274 (TF3 phosphoramidite, OH-reactive) #2271 (TF3 SE) & #2346 (TF3WS SE)
Tide Fluor™ 4 (TF4)	578 nm	602 nm	<i>Alternative to ROX, Texas Red® and Alexa Fluor® 594</i> <ul style="list-style-type: none"> • Strong fluorescence • Photostable 	#2285 (TF4 acid) #2286 (TF4 amine) #2287 (TF4 maleimide, SH-reactive) #2289 (TF4 SE, NH ₂ -reactive) #2300 (TF4 azide, Click chemistry) #2301 (TF4 alkyne, Click chemistry)
Tide Fluor™ 5WS (TF5WS)	649 nm	664 nm	<i>Alternative to Cy5® and Alexa Fluor® 647</i> <ul style="list-style-type: none"> • Strong fluorescence • Photostable 	#2275 (TF5WS azide, Click chemistry) #2276 (TF5WS alkyne, Click chemistry) #2278 (TF5WS acid) #2279 (TF5WS amine) #2280 (TF5WS maleimide, SH-reactive) #2281 (TF5WS SE, NH ₂ -reactive)
Tide Fluor™ 6WS (TF6WS)	682 nm	701 nm	<i>Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680</i> Strong fluorescence Photostable	#2291 (TF6WS acid) #2292 (TF6WS amine) #2293 (TF6WS maleimide, SH-reactive) #2294 (TF6WS SE, NH ₂ -reactive) #2302 (TF6WS azide, Click chemistry) #2303 (TF6WS alkyne, Click chemistry)
Tide Fluor™ 7WS (TF7WS)	756 nm	780 nm	<i>Alternative to Cy7® and Alexa Fluor® 750</i> <ul style="list-style-type: none"> • Strong fluorescence • Photostable 	#2304 (TF7WS azide, Click chemistry) #2305 (TF7WS alkyne, Click chemistry) #2330 (TF7WS acid) #2331 (TF7WS amine) #2332 (TF7WS maleimide, SH-reactive) #2333 (TF7WS SE, NH ₂ -reactive)
Tide Fluor™ 8WS (TF8WS)	785 nm	801 nm	<i>Alternative to IRDye® 800</i> <ul style="list-style-type: none"> • Stronger fluorescence • Higher Photostability 	#2306 (TF8WS azide, Click chemistry) #2307 (TF8WS alkyne, Click chemistry) #2335 (TF8WS acid) #2336 (TF8WS amine) #2337 (TF8WS maleimide, SH-reactive) #2338 (TF8WS SE, NH ₂ -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 μL of deionized water and extract three times with an equal volume of chloroform.
- 2) 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.
- 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 (λ_{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 (A_{max} for dye); The dye-labeled oligonucleotide will have a higher A260:A_{max} 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 μL of 50% formamide, and incubate at 55°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.