

## Amine-Reactive Probes

**Table 1.** Contents and storage information.

Materials	Amount	Concentration	Storage Upon Receipt*	Stability
Reactive dye (lyophilized solids)	Various, see product label	NA	<ul style="list-style-type: none"> <li>• ≤-20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed, dyes are stable for 6–12 months.

\*When stored as directed, dyes are stable for 6–12 months. NA = Not applicable.

**Spectral data:** See the *Appendix*.

### Introduction

Molecular Probes manufactures a wide variety of amine-reactive fluorescent dyes, biotins, and other haptens for conjugation to proteins, amine-modified oligonucleotides or other amine-containing compounds. This manual describes protocols for conjugating amine-reactive compounds to 10 mg of an IgG antibody or to 100 µg of an amine-modified oligonucleotide.

There are three major classes of commonly used reagents to label amines: active esters (which include succinimidyl esters (SE), sulfosuccinimidyl esters (SSE), tetrafluorophenyl esters (TFP) and sulfodichlorophenol esters (SDP)), isothiocyanates (ITC), and sulfonyl chlorides (SC). Of the three major classes, active esters are the preferred chemistry for conjugates as they produce stable carboxamide bonds.<sup>1</sup> Of the four active esters, SDP esters are preferred as they are significantly less susceptible to hydrolysis than succinimidyl esters and TFP esters and therefore can provide greater control or more reaction time in aqueous-based reactions. Other amine-reactive groups include dichlorotriazines, aryl halides, and acyl azides. In addition, we have developed water-soluble amine-reactive forms of some of our popular hydrophobic dyes. These dyes have an amine-reactive 4-sulfo-2,3,5,6-tetrafluorophenyl (STP) ester group and may be a good alternative for labeling biomolecules in the absence of organic solvents. For more details and a complete list of our amine-reactive compounds, please refer to *The Handbook, A Guide to Fluorescent Probes and Labeling Technologies*, available upon request or at our website at [probes.invitrogen.com](http://probes.invitrogen.com). Further information on making bioconjugates can be found in Hermanson, *Bioconjugate Techniques*, Academic Press, 1996 (B7884, available from Molecular Probes).

## Labeling Proteins

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### Materials Required

#### Protein

Amine-reactive reagents may be conjugated with virtually any protein or peptide. The following protocol is optimized for IgG antibodies. The reaction may be scaled for any amount of protein, but the concentration of the protein should be at least 2 mg/mL for optimal results.

#### Reactive Dye

TFP, SDP, and succinimidyl esters are preferred for the conjugation to proteins because they form a very stable amide bond between the dye or hapten and the protein. Isothiocyanates are also commonly used, although the resulting thiourea product has been reported to deteriorate over time. Sulfonyl chlorides and acid halides are more reactive and may allow conjugation to aromatic amines. Sulfonyl chlorides form very stable sulfonamides that can survive complete protein hydrolysis, but since they are more difficult to work with, we do not recommend these for most routine conjugations with proteins.

#### Solvent

For the most part, reactive dyes and haptens are hydrophobic molecules and should be dissolved in high-quality, anhydrous dimethylformamide (DMF) or dimethylsulfoxide (DMSO). *Caution: DMSO should not be used with sulfonyl chlorides, as it reacts with them.*

#### Reaction Buffer

Amine-reactive reagents react with non-protonated aliphatic amine groups, including the amine terminus of proteins and the  $\epsilon$ -amino group of lysines. The  $\epsilon$ -amino group has a  $pK_a$  of around 10.5; in order to maintain this amine group in the non-protonated form, the conjugation must take place in a buffer with slightly basic pH. It is important to avoid buffers that contain primary amines, such as Tris, as these will compete for conjugation with the amine-reactive compound. We recommend the following buffers for conjugation with amine-reactive compounds with proteins:

- 0.1–0.2 M sodium bicarbonate buffer, pH 9.0 for TFP esters, isothiocyanates, sulfonyl chlorides and dichlorotriazines
- 0.1–0.2 M sodium bicarbonate buffer, pH 8.3 for succinimidyl, STP, and SDP esters

More specific labeling of the amine terminus may be achieved using a buffer closer to neutral pH, as the  $pK_a$  of the terminal amine is lower than that of the lysine  $\epsilon$ -amino group.

#### Stop Reagent (Optional)

1.5 M hydroxylamine, pH 8.5, may be used to terminate the reaction and to remove weakly bound probes. To prepare this reagent, dissolve hydroxylamine hydrochloride at 210 mg/mL in distilled water and adjust the pH to 8.5 with 5 M NaOH. Dilute the resulting solution with an equal volume of distilled water. This reagent should be freshly prepared before use (see step 1.5 below).

#### Purification

A typical labeled antibody can be easily separated from free dye using a gel filtration column, such as Sephadex G-25, BioGel P-30 or equivalent, equilibrated with a buffer of your choice. For much smaller or larger proteins, gel filtration media of a suitable molecular weight cutoff should be selected.

## Labeling Protocol

This procedure may be scaled up or down, maintaining the same molar ratios of reagents. It is important to consider that the number and surface position of the amines will vary greatly among proteins and even among different IgGs, as will the reactivity of the dyes. We therefore recommend that whenever possible three different degrees of labeling be tried, using three different molar ratios of the reactive reagent to protein, and that future protocols be based on the amount of reagent that gives the most satisfactory results for your specific protein. Reviews by Brinkley and by Haugland provide comprehensive surveys of procedures and reagents for protein conjugate preparation.<sup>2,3</sup>

**1.1 Dissolve ~10 mg of the protein in 1 mL of 0.1 M sodium bicarbonate buffer.** The protein concentration in the reaction should usually be 5–20 mg/mL. The kinetics and success of the reaction are highly concentration dependent. Concentrations lower than 2 mg/mL will greatly decrease the efficiency of the reaction.

Protein solutions must be free of any amine-containing substances such as Tris, glycine, or ammonium ions. Antibodies that have been previously dissolved in buffers containing amines can be dialyzed against 10–20 mM phosphate-buffered saline (PBS), and the desired pH for the reaction can be obtained by adding 0.1 mL of 1 M sodium bicarbonate buffer (pH 8.3–9.0) for each mL of antibody solution. The presence of low concentrations of sodium azide (<3 mM) or thimerosal (<1 mM) will not interfere with the conjugation reaction.

**1.2 Dissolve the amine-reactive compound in DMF or (except for sulfonyl chlorides) DMSO at 10 mg/mL.** For a typical reaction, 5 mg of dye can be dissolved in 0.5 mL of DMF or DMSO. It is important that the dye be dissolved immediately before starting the reaction as reactive compounds are not very stable in solution. Stock solutions of dyes with an STP ester reactive group can be prepared in deionized water. Briefly sonicate or vortex.

**1.3 While stirring or vortexing the protein solution (step 1.1), slowly add 50–100 µL of the reactive dye solution (step 1.2).** This volume corresponds to 0.5–1 mg of amine-reactive dye. In general, about one-fourth to one-third of the reactive dye will conjugate to the protein. This percentage may be higher with isothiocyanates. Variations due to the different reactivities of both the protein and the labeling reagent may occur, which may necessitate optimization of the dye-to-protein ratio used in the reaction.

**1.4 Incubate the reaction for 1 hour at room temperature with continuous stirring.** For sulfonyl chlorides, incubate at 4°C, with continuous stirring.

**1.5 Optional: Stop the reaction by adding 0.1 mL of freshly prepared 1.5 M hydroxylamine, pH 8.5.** For Rhodol Green™ and Rhodamine Green™ conjugates, the hydroxylamine-containing reaction should be incubated overnight at 4°C. Otherwise, the hydroxylamine-containing reaction should be incubated for one hour at room temperature.

Treatment with hydroxylamine at this stage is required to remove the trifluoroacetyl protecting groups of Rhodamine Green™ (R6112) succinimidyl ester. Hydroxylamine may also remove dye from unstable conjugates with tyrosine, serine, threonine and histidine.

**1.6 Separate the conjugate from unreacted labeling reagent.**

We recommend using Sephadex G, BioGel P or equivalent gel filtration media of the appropriate molecular weight cutoff. A 10 × 300 mm column should be equilibrated PBS or buffer of choice. The excluded fraction, corresponding to the first fluorescent band to elute, will be the conjugate. If you are conjugating a dilute antibody, to avoid further dilution you may want to purify the conjugate by extensive dialysis.

Note: Microdialysis apparatus for small volumes of proteins, e.g., 10–500 µL can be obtained from Pierce Chemical Company ([www.piercenet.com](http://www.piercenet.com)) and Spectrum Laboratories ([www.spectrapor.com](http://www.spectrapor.com)).

If you prefer to purify your conjugate by column chromatography, after elution, add bovine serum albumin (BSA) or any other stabilizer of choice to a final concentration of 1–10 mg/mL to prevent denaturation.

**1.7 Storage of protein conjugate.** In general, conjugates should be stored under the same conditions used for the parent protein. For storage in solution at 2–6°C, sodium azide (2 mM final concentration) should be added as a preservative. Since azide is an inhibitor of horseradish peroxidase (HRP), thimerosal should be substituted as a preservative for conjugates that are derived from HRP or that will be used for experiments in which HRP is present. Removal of preservatives prior to use may be necessary to avoid inhibitory effects in applications in which conjugates are added to live cell specimens.

## Determining the Degree of Labeling

You may need to optimize the labeling efficiency to achieve the desired results in your application. The relative efficiency of a labeling reaction can be determined by measuring the absorbance of the protein at 280 nm and the absorbance of the dye at its absorbance maximum ( $\lambda_{\text{max}}$ ). Using the Beer-Lambert law:  $A = \epsilon \times \text{path length} \times \text{concentration}$ , where  $\epsilon$  is the extinction coefficient in  $\text{cm}^{-1}\text{M}^{-1}$ , one can calculate the approximate number of dye molecules per protein molecule. A correction needs to be made for the absorbance of the dye at 280 nm. In the case of a biotinylated conjugate, the degree of labeling can be measured with HABA reagent as described in the protocol of our FluoReporter® Biotin-XX Protein Labeling Kit (F2610) and elsewhere.<sup>4</sup>

**2.1 Measure the absorbance of the protein–dye conjugate at 280 nm ( $A_{280}$ ) and at the  $\lambda_{\text{max}}$  for the dye ( $A_{\text{max}}$ ).** Dilute the protein–dye conjugate to approximately 0.1 mg/mL. Dilute only as much as you need to make the measurement. The  $\lambda_{\text{max}}$  values for commonly used fluorophores are given in the *Appendix*. Please consult *The Handbook, A Guide to Fluorescent Probes and Labeling Technologies*, or our website at [probes.invitrogen.com](http://probes.invitrogen.com) for information about other dyes.

**2.2 Determine the concentration of the protein in mg/mL.**

a. Correct for the contribution of the dye to the absorbance at  $A_{280}$ .

$$A_{\text{protein}} = A_{280} - A_{\text{max}} (\text{CF})$$

$$\text{CF} = \frac{A_{280 \text{ free dye}}}{A_{\text{max free dye}}}$$

CF values for commonly used fluorophores are listed in the *Appendix*.

b. Calculate the protein concentration assuming 1.4  $A_{\text{protein}}$  units = 1 mg/mL. This value is correct for IgG antibodies, but may be different for your protein.

**2.3 Calculate the degree of labeling (D.O.L.):**

$$\text{DOL} = \frac{A_{\text{max}} \times \text{MW}}{[\text{protein}] \times \epsilon_{\text{dye}}}$$

where MW = the molecular weight of the protein,  $\epsilon_{\text{dye}}$  = the extinction coefficient of the dye at its absorbance maximum and the protein concentration is in mg/mL.

## Labeling Amine-Modified Oligonucleotides

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### Materials Required

#### Oligonucleotide

The oligonucleotide must be synthesized with an amine group on the 5' end. The following protocol has been optimized for use with 100 µg of oligonucleotide.

#### Reactive Dye

Succinimidyl esters are preferred for the conjugation to oligonucleotides because they are easy to use and form a very stable amide bond between the dye or hapten and the amine-modified oligonucleotide. For DNA sequencing applications, the BODIPY® dyes are useful because they are isomerically pure and cause little perturbation to the mobility of DNA fragments.<sup>5</sup> We also offer 5-FAM, 6-TAMRA, 6-ROX, 6-HEX, 6-TET and 6-JOE, the traditional fluorophores used in oligonucleotide labeling and automated DNA sequencing applications.<sup>6-9</sup> The Alexa Fluor® series of dyes provides very bright, photostable fluorescence; however, note that the fluorescence of the Alexa Fluor® 633 dye appears to be quenched by nucleic acids, so this dye should not be used to label oligonucleotides.

#### Solvent

For the most part, reactive dyes and haptens are hydrophobic molecules and should be dissolved in high-quality, dimethylsulf-oxide (DMSO) before reaction with amine-modified oligonucleotides.

#### Reaction Buffer

Amine-reactive reagents will react with the non-protonated amine group on the modified oligonucleotide. In order to maintain this amine group in the non-protonated form, the conjugation must take place in a buffer with slightly basic pH. For optimal results we recommend using a tetraborate buffer at pH 8.5, rather than the bicarbonate buffers recommended for protein conjugations. It is important to avoid buffers that contain primary amines, such as Tris, as these will compete for conjugation with the amine-reactive compound.

### Labeling Protocol

The protocol has been optimized for labeling 100 µg of an 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.

**3.1 Purify 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 dH<sub>2</sub>O 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 vacuum.
- Dissolve the dry pellet in dH<sub>2</sub>O 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.

**3.2 Prepare 0.1 M sodium tetraborate, pH 8.5 labeling buffer.** Make a 0.1 M sodium tetraborate buffer by dissolving 0.038 g of sodium tetraborate decahydrate for every mL of water. Adjust pH with HCl to 8.5. This labeling buffer should be made as close as possible to the time of labeling. Alternatively, it may be divided into small aliquots and frozen immediately for long-term storage. Exposure of this solution to air for a long time will result in carbon dioxide absorption, which will change the pH of the buffer.

**3.3 Dissolve 250 µg of the amine-reactive compound in 14 µL DMSO.** Allow amine-reactive compound to come to room temperature before opening the vial. Do not heat. For dinitrophenyl-X (DNP-X) succinimidyl ester, use 160 µg for 100 µg of oligonucleotide. For fluorescein, tetramethylrhodamine, Marina Blue®, FAM, or TAMRA succinimidyl ester, use 200 µg for 100 µg of oligonucleotide. Dissolve the material by pipetting up and down, washing the sides of the vial. Texas Red® compounds tend to precipitate easily and may require longer times to completely dissolve.

It is important that the amine-reactive label be freshly prepared for each labeling reaction as reactive compounds are not stable in solution.

**3.4 To the vial containing the reactive label in DMSO, add:**

- 7 µL dH<sub>2</sub>O
- 75 µL labeling buffer (step 3.2)
- 4 µL of a 25 µg/µL oligonucleotide stock solution (step 3.1)

The reaction mixture may have a grainy appearance, but this should 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. The reaction may be scaled up or down as long as the concentration of each component is not changed. Do not add more dye than recommended, as excess dye will not improve the labeling efficiency and may make the purification more difficult.

**3.5 Incubate the reaction for at least six hours (or overnight if more convenient) at room temperature.** Place the vial on a shaker oscillating at low speed or 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. Allowing the incubation to proceed overnight does not necessarily result in a greater labeling efficiency.

## Purifying the Labeled Oligonucleotide

Following the reaction, the labeling mixture contains labeled oligonucleotide, unlabeled oligonucleotide, and unincorporated dye (or biotin or DNP). 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 oligonucleotide 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.
- Some reactions may benefit from a second ethanol precipitation in order to adequately eliminate unreacted dye. In particular, when using tetramethylrhodamine (TAMRA), you should redissolve the oligonucleotide pellet in dH<sub>2</sub>O and repeat the ethanol precipitation. This extra ethanol precipitation step is necessary because the tetramethylrhodamine labeling reagent has a tendency to adhere nonspecifically to the oligonucleotide. Similarly, a second ethanol precipitation is often appropriate when using the Texas Red<sup>®</sup>-X, BODIPY<sup>®</sup> 564/570, BODIPY<sup>®</sup> 581/591 or BODIPY<sup>®</sup> 630/650-X dyes.

### Purifying by HPLC

Labeled oligonucleotides can be purified by reverse-phase HPLC using a standard analytical (4.6 × 250 mm) C8 column. Dissolve the pellet from the ethanol precipitation in 0.1 M TEAA (triethylammonium acetate). Load the dissolved pellet onto the column in 0.1 M TEAA and run a linear 5–65% acetonitrile gradient over 30 minutes. This gradient is a 2% increase in acetonitrile per minute. For oligonucleotides labeled with very hydrophobic dyes, like Texas Red<sup>®</sup> dye, you can achieve good separation running a faster gradient with up to a 3% increase per minute. For separation of oligonucleotides labeled with more hydrophilic dyes, like Marina Blue<sup>®</sup> dye, run a slower gradient, about 1% increase in acetonitrile per minute. In all cases, the unlabeled oligonucleotide will migrate fastest, followed by the labeled oligonucleotide and finally the free dye. For more details, please refer to Oliver R.W.A., *HPLC of Macromolecules: A Practical Approach*, IRL Press (1989).

### Purifying 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. Fluorophore-labeled oligonucleotides will show fluorescence when illuminated with UV light. Cut out the band containing the labeled oligonucleotide and purify 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).

## References

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1. *Bioconjug Chem* 6, 447 (1995); 2. *Bioconjug Chem* 3, 2 (1992); 3. *Methods Mol Biol* 45, 205 (1995); 4. *Methods Enzymol* 18A, 418 (1970); 5. *Science* 271, 1420 (1996); 6. *Anal Biochem* 238, 165 (1996); 7. *Anal Biochem* 223, 39 (1994); 8. *Nucleic Acids Res* 20, 2471 (1992); 9. *J Clin Microbiol* 37, 165 (1999).

## Appendix

Spectral characteristics of common dyes. Values for other dyes may be found at our website ([probes.invitrogen.com](http://probes.invitrogen.com)).

Dye	$\lambda_{\text{Max}}^*$	Em *	$\epsilon^\dagger$	CF <sub>280</sub> ‡
Alexa Fluor® 350	346	442	19,000	0.19
Alexa Fluor® 405	401	421	34,500	0.70
Alexa Fluor® 430	434	531	16,000	0.28
Alexa Fluor® 488	495	519	71,000	0.11
Alexa Fluor® 500	502	525	71,000	0.18
Alexa Fluor® 514	517	542	80,000	0.18
Alexa Fluor® 532	532	554	81,000	0.09
Alexa Fluor® 546	556	573	104,000	0.12
Alexa Fluor® 555	555	565	150,000	0.08
Alexa Fluor® 568	578	603	91,300	0.46
Alexa Fluor® 594	590	617	73,000	0.56
Alexa Fluor® 610-X	602	624	132,000	0.44
Alexa Fluor® 633	632	647	100,000	0.55
Alexa Fluor® 647	650	665	239,000	0.03
Alexa Fluor® 660	663	690	110,000	0.10
Alexa Fluor® 680	679	702	184,000	0.05
Alexa Fluor® 700	702	723	192,000	0.07
Alexa Fluor® 750	749	775	240,000	0.04
Alexa Fluor® 790	785	810	260,000	0.08
AMCA-X	346	442	19,000	0.19
BODIPY® 630/650	632	640	100,000	0.10
BODIPY® 650/665	651	660	100,000	0.13
BODIPY® FL	504	513	68,000	0.04
BODIPY® TMR	535	574	50,000	0.05
BODIPY® TR	588	617	45,000	0.22
Cascade Blue®	400	420	28,000	0.65
Dintrophenyl	349	NA	18,000	0.18
Fluorescein (FAM)	494	518	68,000 §	0.30
HEX	535	556	98,000	0.15
JOE **	520	584	75,000	0.25
Marina Blue®	365	460	20,000	0.30
Oregon Green® 488	496	524	70,000 §	0.12
Oregon Green® 514	511	530	70,000	0.19
Pacific Blue™	416	451	46,000	0.20
Pacific Orange™	400	551	24,500	0.60
Rhodamine Green™	505	527	68,000 §	0.19
QSY® 7	560	NA	90,000	0.22
QSY® 9	562	NA	85,000	0.23
QSY® 21	660	NA	89,000	0.32
QSY® 35	472	NA	23,500	0.19
ROX	575	602	82,000	0.16
Rhodamine Red™	570	590	120,000	0.17
TET	521	536	99,000	0.13
Tetramethylrhodamine (TAMRA)	555	580	65,000	0.30
Texas Red®	595	615	80,000	0.18

\* Absorbance and fluorescence emission maxima, in nm. † Extinction coefficient at  $\epsilon_{\text{Max}}$  in  $\text{cm}^{-1}\text{M}^{-1}$ . ‡ Correction factor ( $A_{280}$  free dye /  $A_{\text{Max}}$  free dye). § Measured at pH 8.0. \*\* Measured at pH 12.0. NA = Not applicable.

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