Labeling Alkyne-Modified Biomolecules with Fluorescent Dye Azides

Labeling Oligonucleotides with Dye Azides

1. Prepare the following stock solutions:
    - 200 mM THPTA [tris(3-hydroxypropyltriazolylmethyl)amine)] in water
    - 100 mM $CuSO_4$ in water
    - Alkyne-modified oligo in water (as concentrated as possible, e.g., >10 mg/mL)
    - 100 mM sodium ascorbate in water
    - 10 mM dye azide in DMSO or water (see our website for recommended solvent)
2. Mix and vortex well $CuSO_4$ with THPTA in a 1:2 ratio for several minutes before the reaction. This working solution is stable for several weeks when frozen.
3. To the alkyne-modified oligo solution, add an excess of dye azide (2-5 equivalents by molar ratio).
4. Add 5 equivalents of THPTA/$CuSO_4$ working solution (from Step 1)
5. Add 10-30 equivalents of sodium ascorbate.
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Ethanol-precipitate the oligo or purify it by your desired method (e.g., HPLC).

Labeling Peptides with Dye Azides

1. Prepare the following stock solutions:
    - 200 mM THPTA ligand in water
    - 100 mM $CuSO_4$ in water
    - Alkyne-modified peptide in water or DMF (depending on your peptide solubility, >10 mg/mL if possible)
    - 100 mM sodium ascorbate in water
    - 10 mM dye azide in DMSO or water (see our website for recommended solvent)
2. Incubate $CuSO_4$ with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
3. To the alkyne-modified peptide solution, add an excess of dye azide (5-10 equivalents by molar ratio).
4. Add 5-10 equivalents of THPTA/$CuSO_4$.
5. Add 10-20 equivalents of sodium ascorbate.
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired peptide by HPLC.

Labeling Small Organic Alkyne Molecules with Dye Azides

1. Prepare the following stock solutions:
    - 200 mM THPTA ligand in water
    - 100 mM $CuSO_4$ in water
    - Alkyne compound in water or DMF (depending on your compound solubility, >10 mg/mL if possible)
    - 100 mM sodium ascorbate in water
    - 10 mM dye azide in DMSO or water (see our website for recommended solvent)
2. Incubate $CuSO_4$ with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
3. To the alkyne solution, add an excess of dye azide (5-10 equivalents by molar ratio).
4. Add 25 equivalents of THPTA/$CuSO_4$.
5. Add 50 equivalents of sodium ascorbate.
6. Stir the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired molecule by chromatography or other methods.

Labeling Biopolymers with Dye Azides

1. Prepare the following stock solutions:
    - 200 mM THPTA ligand in water
    - 100 mM $CuSO_4$ in water
    - Alkyne-modified biopolymer in water (as concentrated as possible, e.g., >5 mg/mL)
    - 100 mM sodium ascorbate in water
    - 10 mM dye azide in DMSO or water (see our website for recommended solvent)
2. Incubate $CuSO_4$ with THPTA ligand in a 1:2 ratio several minutes before the reaction. This solution is stable for several weeks when frozen.
3. To the alkyne-modified biopolymer solution, add an excess of dye azide (Loading ratio: 5-20 dye azide/alkyne).
4. Add 5 molar equivalents (referenced to dye azide) of THPTA/$CuSO_4$.
5. Add 10 equivalents of sodium ascorbate (referenced to dye azide).
6. Stir, vortex or shake the reaction mixture at room temperature for 30-60 minutes.
7. Purify your desired molecule by gel filtration or dialysis.

Labeling Cells, Cell Lysates or Biological Samples with Dye Azides or Dye Alkynes

1. Prepare the following click solutions:
    - 100 mM THPTA ligand in aqueous buffer or water
    - 20 mM CuSO4 in water
    - 300 mM sodium ascorbate in water
    - 2.5 mM alkyne or azide labeling reagent in water or DMSO
2. For each azide- or alkyne-modified cell or cell lysate sample, add the following reagents to a 1.5 mL microfuge tube, then vortex briefly to mix.
    - 50 µL cell or cell lysate sample
    - 50 µL PBS buffer
    - 50 µL of 5 mM corresponding dye azide (or dye alkyne) detection reagent in DMSO or water
3. Add 10 µL of 100 mM THPTA solution, vortex briefly to mix.
4. Add 10 µL of 20 mM CuSO4 solution, vortex briefly to mix.
5. Add 10 µL of 300 mM sodium ascorbate solution to initiate click reaction, vortex briefly to mix.
6. Protect reaction from light and allow click reaction to incubate for 30 minutes at room temperature.
7. Cells or cell lysates are now click labeled and ready for downstream processing and/or analysis.

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 comprised 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_2H	+ 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_2H	+ 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_2H	+ 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_2H	+ 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_2H	+ 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_2H	+ 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

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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_2H	+ 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_2H	+ 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_2H	+ 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_2H	+ 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	*Alternative to EDANS* • 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	*Alternative to FAM, FITC and Alexa Fluor® 488* • 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	*Alternative to Cy3® and Alexa Fluor® 555* • 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	*Alternative to ROX, Texas Red® and Alexa Fluor® 594* • 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	*Alternative to Cy5® and Alexa Fluor® 647* • 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	*Alternative to Cy5.5®, IRDye® 700 and Alexa Fluor® 680* 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	*Alternative to Cy7® and Alexa Fluor® 750* • 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	*Alternative to IRDye® 800* • 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 µg/µL. This amine-modified oligonucleotide stock solution may be immediately used or stored frozen at ≤–15°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 (λmax) 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 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.