

**Product Information: ATTO-Reagents for Click Chemistry
(Alkyne- or azide-modified ATTO-label)**

| Compound | Storage information | Shelf Life |
|--|---|--|
| Alkyne- or azide-modified reactive label Lyophilized or crystalline solid | Freeze upon receipt < -20°C Protect from light and moisture | When stored as indicated, ATTO reagents for Click Chemistry are stable for at least 3 years. |
| For optical properties see table on page 3. | | |

Introduction:

The term “**Click Chemistry**” describes chemical reactions that are able to quickly and reliably generate substances by joining together small units. One of the most popular reactions within the Click Chemistry concept is the copper (I) catalyzed Huisgen azide alkyne cycloaddition forming a covalent linking unit (triazole) between the label and the target molecule (Figure 1).

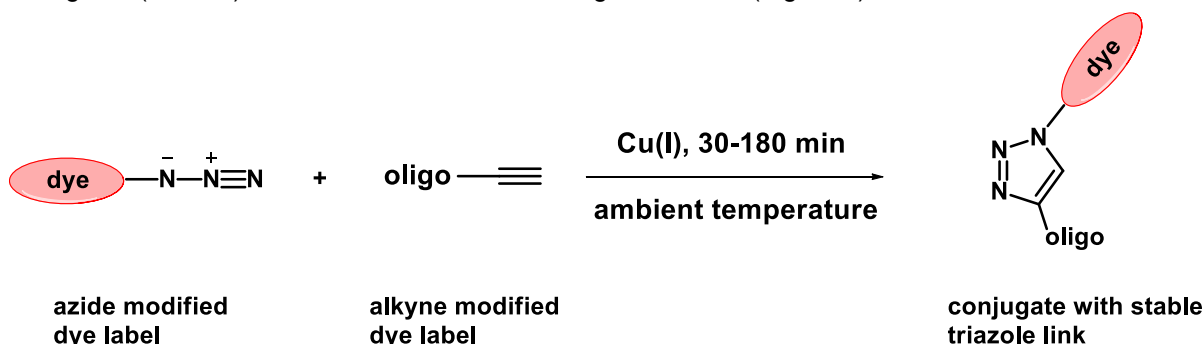


Figure 1: Cu(I) catalysed alkyne-azide “Click Reaction”. The azide and the alkyne residues are interchangeable e.g. the fluorophore can be labeled with an azide moiety and the target molecule could carry the alkyne functionality.

The alkyne and azide groups are biologically unique and therefore the click reaction is very selective and specific. The fluorescent labeled click conjugates can be easily detected with high sensitivity and low background, unlike conventional labeling reactions using succinimidyl ester or maleimides which react with amines and thiols, which are common functionalities in biological environment.

ATTO-TEC offers a large variety of alkyne- or azide-modified fluorophores for Click Chemistry.

Protocol for Oligonucleotide Labeling via Click Chemistry

General Remarks

The following protocol describes the labeling procedure for 5 nmol of a single alkyne modified oligonucleotide.

The reaction is most efficient if the azide and alkyne are dissolved in a minimal amount of solvent and the solutions are of high concentration.

The reaction can be accelerated by raising the temperature and is generally finished in 30 min at around 40 – 45 °C.

Required Materials

- **Solution A:** Dissolve the azide- or alkyne-modified oligonucleotide in the appropriate amount of water to obtain a 2 mM solution and centrifuge shortly.
- **Solution B:** Dissolve 1.0 mg of the click reagent (azide- or alkyne-modified ATTO-dye) in the appropriate amount of DMSO/t-BuOH-solution 1:1 to obtain a 50 mM stock-solution. In case of azide or alkyne of ATTO 725, ATTO 740, ATTO 647, and ATTO 610 we recommend using ACN/t-BuOH.
- **Solution C:** Click Solution: Dissolve 54 mg TBTA (tris[(1-benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine) in 1 ml DMSO/t-BuOH 3:1 for a 0.1 M solution. The solution can be stored at -20 °C.
- **Solution D:** Dissolve 1 mg CuBr in 70 µl DMSO/t-BuOH 3:1 to obtain a 0.1 M solution.
NOTE: This solution must be freshly prepared and cannot be stored!
- **Solution E:** The final click solution is prepared by quickly adding 1 volume of **Solution D** to 2 volumes of **Solution C**.

Conjugate Preparation

In general, the labeling reaction works more efficiently with concentrated solutions of alkynes (e.g. oligo) and azides (dye label). In the case the reaction does not work in water, rising the pH by performing the reaction in Tris-HCl (50 mM) at pH 8.3 might be helpful.

- Pipette 5 µl of **Solution A** (10 nmol of oligonucleotide) in a 0.5 reaction vial.
- Add 1 – 2 µl of **Solution B** (50 – 100 nmol; 5 – 10 eq.) to the reaction vial.
- Pipette the correct amount of **Solution B** corresponding to a 2 – 10 times molar excess of the alkyne-or azide modified ATTO-dye into the reaction vial.
- 3 µl of freshly prepared **Solution E** is added and the reaction vial is thoroughly mixed by shaking at 25 °C for 3 h. As previously mentioned, by rising the temperature to 40 – 45 °C the reaction is generally finished in 30 minutes.

Conjugate Purification – Removal of Excess Reagents

- Add 100 µl of a 0.3 M NaOAc solution and precipitate the oligonucleotide by adding 1 ml cold ethanol (containing 5 % diethylether; -20 °C). Centrifuge for at least 20 min at 6000 rpm or higher. Remove the supernatant and wash the residue with 100 µl cold ethanol (containing 5 % diethyl ether; -20 °C). Centrifuge again for at least 10 min and remove the supernatant. Dry the residue on air.

Storage of the Conjugate

In general, conjugates should be stored under the same conditions used for the unlabeled oligonucleotide. For long-term storage we recommend freezing at -20 °C. Protect dye conjugates from light as much as possible.

Table 1: Properties of ATTO-dye labeled azides:

| Dye | Order # | | MW | M ⁺ | λ_{abs} | λ_{em} | ϵ_{max} | CF ₂₆₀ | CF ₂₈₀ |
|-------------|---------------|---------------|------|----------------|------------------------|-----------------------|-------------------------|-------------------|-------------------|
| | 1 mg | 5 mg | | | | | | | |
| ATTO 390 | AD 390-101 | AD 390-105 | 544 | 544 | 390 | 476 | 24000 | 0.46 | 0.09 |
| ATTO 425 | AD 425-101 | AD 425-105 | 602 | 602 | 439 | 485 | 45000 | 0.19 | 0.17 |
| ATTO 430LS | AD 430LS-101 | AD 430LS-105 | 789 | 767 | 436 | 545 | 32000 | 0.32 | 0.22 |
| ATTO 465 | AD 465-101 | AD 465-105 | 610 | 496 | 453 | 506 | 75000 | 1.09 | 0.48 |
| ATTO 488 | AD 488-101 | AD 488-105 | 904 | 790 | 500 | 520 | 90000 | 0.22 | 0.09 |
| ATTO 490LS | AD 490LS-101 | AD 490LS-105 | 896 | 874 | 495 | 658 | 40000 | 0.39 | 0.21 |
| ATTO Rho110 | AD Rho110-101 | AD Rho110-105 | 744 | 630 | 507 | 531 | 90000 | 0.21 | 0.14 |
| ATTO 514 | AD 514-101 | AD 514-105 | 1068 | 954 | 511 | 532 | 11500 | 0.21 | 0.07 |
| ATTO 520 | AD 520-101 | AD 520-105 | 681 | 567 | 517 | 538 | 11000 | 0.16 | 0.20 |
| ATTO 532 | AD 532-101 | AD 532-105 | 960 | 846 | 532 | 552 | 11500 | 0.20 | 0.09 |
| ATTO Rho6G | AD Rho6G-101 | AD Rho6G-105 | 828 | 714 | 533 | 557 | 11500 | 0.19 | 0.16 |
| ATTO 540Q | AD 540Q-101 | AD 540Q-105 | 873 | 759 | 543 | | 10500 | 0.27 | 0.26 |
| ATTO 542 | AD 542-101 | AD 542-105 | 1228 | 1114 | 542 | 562 | 12000 | 0.18 | 0.08 |
| ATTO 550 | AD 550-101 | AD 550-105 | 908 | 794 | 554 | 576 | 12000 | 0.23 | 0.10 |
| ATTO 565 | AD 565-101 | AD 565-105 | 811 | 711 | 564 | 590 | 12000 | 0.27 | 0.12 |
| ATTO Rho12 | AD Rho12-101 | AD Rho12-105 | 964 | 851 | 577 | 600 | 12000 | 0.29 | 0.09 |
| ATTO Thio12 | AD Thio12-101 | AD Thio12-105 | 816 | 702 | 582 | 607 | 11000 | 0.11 | 0.37 |
| ATTO Rho101 | AD Rho101-101 | AD Rho101-105 | 890 | 790 | 587 | 609 | 12000 | 0.18 | 0.17 |
| ATTO 590 | AD 590-101 | AD 590-105 | 905 | 791 | 593 | 622 | 12000 | 0.39 | 0.43 |
| ATTO 594 | AD 594-101 | AD 594-105 | 1119 | 1006 | 603 | 626 | 12000 | 0.22 | 0.50 |
| ATTO 633 | AD 633-101 | AD 633-105 | 866 | 752 | 630 | 651 | 13000 | 0.04 | 0.05 |
| ATTO 643 | AD 643-101 | AD 643-105 | 1149 | 1036 | 643 | 665 | 15000 | 0.05 | 0.04 |
| ATTO 647N | AD 647N-101 | AD 647N-105 | 960 | 846 | 646 | 664 | 15000 | 0.04 | 0.03 |
| ATTO 655 | AD 655-101 | AD 655-105 | 842 | 728 | 663 | 680 | 12500 | 0.24 | 0.08 |
| ATTO 665 | AD 665-101 | AD 665-105 | 937 | 823 | 662 | 680 | 16000 | 0.07 | 0.06 |
| ATTO 680 | AD 680-101 | AD 680-105 | 839 | 726 | 681 | 698 | 12500 | 0.30 | 0.17 |
| ATTO 700 | AD 700-101 | AD 680-105 | 880 | 766 | 700 | 716 | 12500 | 0.26 | 0.41 |
| ATTO 725 | AD 725-101 | AD 725-105 | 729 | 616 | 728 | 751 | 12000 | 0.08 | 0.06 |
| ATTO 740 | AD 740-101 | AD 740-105 | 782 | 668 | 743 | 763 | 12000 | 0.07 | 0.07 |

Table 2: Properties of ATTO-dye labeled alkyne:

| Dye | Order # | | MW | M ⁺ | λ_{abs} | λ_{em} | ϵ_{max} | CF ₂₆₀ | CF ₂₈₀ |
|------------|--------------|--------------|-----|----------------|------------------------|-----------------------|-------------------------|-------------------|-------------------|
| | 1 mg | 5 mg | | | | | | | |
| ATTO 390 | AD 390-141 | AD 390-145 | 495 | 381 | 390 | 476 | 24000 | 0.46 | 0.09 |
| ATTO 488 | AD 488-141 | AD 488-145 | 741 | 627 | 500 | 520 | 90000 | 0.22 | 0.09 |
| ATTO 514 | AD 514-141 | AD 514-145 | 905 | 791 | 511 | 532 | 11500 | 0.21 | 0.07 |
| ATTO 532 | AD 532-141 | AD 532-145 | 797 | 683 | 532 | 552 | 11500 | 0.20 | 0.09 |
| ATTO Rho6G | AD Rho6G-141 | AD Rho6G-145 | 651 | 551 | 533 | 557 | 11500 | 0.19 | 0.16 |
| ATTO 550 | AD 550-141 | AD 550-145 | 731 | 631 | 554 | 576 | 12000 | 0.23 | 0.10 |
| ATTO 565 | AD 565-141 | AD 565-145 | 648 | 548 | 564 | 590 | 12000 | 0.27 | 0.12 |
| ATTO 590 | AD 590-141 | AD 590-145 | 742 | 628 | 593 | 622 | 12000 | 0.39 | 0.43 |
| ATTO 594 | AD 594-141 | AD 594-145 | 956 | 843 | 603 | 626 | 12000 | 0.22 | 0.50 |
| ATTO 633 | AD 633-141 | AD 633-145 | 703 | 589 | 630 | 651 | 13000 | 0.04 | 0.05 |
| ATTO 643 | AD 643-141 | AD 643-145 | 987 | 873 | 643 | 665 | 15000 | 0.05 | 0.04 |
| ATTO 647N | AD 647N-141 | AD 647N-145 | 783 | 683 | 646 | 664 | 15000 | 0.04 | 0.03 |
| ATTO 655 | AD 655-141 | AD 655-145 | 679 | 565 | 663 | 680 | 12500 | 0.24 | 0.08 |
| ATTO 680 | AD 680-141 | AD 680-145 | 677 | 563 | 681 | 698 | 12500 | 0.30 | 0.17 |
| ATTO 700 | AD 700-141 | AD 700-145 | 717 | 603 | 700 | 716 | 12500 | 0.26 | 0.41 |

MW: molecular weight of the dye including counterions in g/mol; M⁺: molecular weight of dye cation (HPLC_MS acetonitrile/water 0.1 vol-% trifluoroacetic acid); λ_{abs} : longest wavelength absorption maximum in nm; λ_{em} : fluorescence maximum in nm; ϵ_{max} : molar decadic extinction coefficient at the longest-wavelength absorption maximum in M⁻¹ cm⁻¹; CF₂₆₀ = $\epsilon_{260}/\epsilon_{\text{max}}$; CF₂₈₀ = $\epsilon_{280}/\epsilon_{\text{max}}$;

Detailed information on each individual dye including risk and safety data can be downloaded from our website at www.atto-tec.com.

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ATTO-TEC products are high-quality reagents intended for research purposes only. These compounds must be used by, or under the direct supervision of, technically qualified individuals experienced in handling potentially hazardous chemicals. We refer to the **Material Safety Data Sheet (MSDS)** provided with each product.

Additional information on **ATTO-TEC** and its entire product range is available on our website www.atto-tec.com. For further questions contact us directly by e-mail or fax.