ATTO-TEC

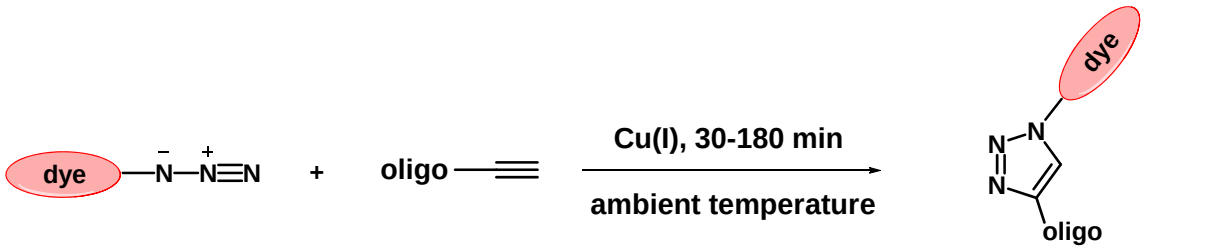
Revised: January 27, 2020

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).



azide modified dye label **alkyne modified dye label** **conjugate with stable triazole link**

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 sulfhydryls, which are common functionalities in biological environment.
ATTO-TEC offers a large variety of alkyne- or azide-modified fluorophores for Clikck 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 to use 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 to freeze at -20 °C. Protect dye conjugates from light as much as possible.

Table 1: Properties of **ATTO**-dye labeled azides:

Dye	Order # 1 mg	Order # 5 mg	MW	M^+	λ_{abs}	λ_{em}	ε_{max}	CF_{260}	CF_{280}
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 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 514	AD 514-101	AD 514-105	1068	954	511	532	115000	0.21	0.07
ATTO 520	AD 520-101	AD 520-105	681	567	517	538	110000	0.16	0.20
ATTO 532	AD 532-101	AD 532-105	960	846	532	552	115000	0.20	0.09
ATTO Rho6G	AD Rho6G-101	AD Rho6G-105	828	714	533	557	115000	0.19	0.16
ATTO 540Q	AD 540Q-101	AD 540Q-105	873	759	543		105000	0.27	0.26
ATTO 542	AD 542-101	AD 542-105	1228	1114	542	562	120000	0.18	0.08
ATTO 550	AD 550-101	AD 550-105	908	794	554	576	120000	0.23	0.10
ATTO 565	AD 565-101	AD 565-105	825	711	564	590	120000	0.27	0.12
ATTO Rho12	AD Rho12-101	AD Rho12-105	964	851	577	600	120000	0.29	0.09
ATTO Rho101	AD Rho101-101	AD Rho101-105	890	790	587	609	120000	0.18	0.17
ATTO 590	AD 590-101	AD 590-105	905	791	593	622	120000	0.39	0.43
ATTO 594	AD 594-101	AD 594-105	1119	1006	603	626	120000	0.22	0.50
ATTO 633	AD 633-101	AD 633-105	866	752	630	651	130000	0.04	0.05
ATTO 643	AD 643-101	AD 643-105	1149	1036	643	665	150000	0.05	0.04
ATTO 647**N**	AD 647N-101	AD 647N-105	960	846	646	664	150000	0.04	0.03
ATTO 655	AD 655-101	AD 655-105	842	728	663	680	125000	0.24	0.08
ATTO 665	AD 665-101	AD 665-105	937	823	662	680	160000	0.07	0.06
ATTO 680	AD 680-101	AD 680-105	839	726	681	698	125000	0.30	0.17
ATTO 700	AD 700-101	AD 680-105	880	766	700	716	125000	0.26	0.41
ATTO 725	AD 725-101	AD 725-105	729	616	728	751	120000	0.08	0.06
ATTO 740	AD 740-101	AD 740-105	782	668	743	763	120000	0.07	0.07

Table 2: Properties of **ATTO**-dye labeled alkyne:

Dye	Order # 1 mg	Order # 5 mg	MW	M^+	λ_{abs}	λ_{em}	ε_{max}	CF_{260}	CF_{280}
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	115000	0.21	0.07
ATTO 532	AD 532-141	AD 532-145	797	683	532	552	115000	0.20	0.09
ATTO Rho6G	AD Rho6G-141	AD Rho6G-145	651	551	533	557	115000	0.19	0.16
ATTO 550	AD 550-141	AD 550-145	731	631	554	576	120000	0.23	0.10
ATTO 565	AD 565-141	AD 565-145	648	548	564	590	120000	0.27	0.12
ATTO 590	AD 590-141	AD 590-145	742	628	593	622	120000	0.39	0.43
ATTO 594	AD 594-141	AD 594-145	956	843	603	626	120000	0.22	0.50
ATTO 633	AD 633-141	AD 633-145	703	589	630	651	130000	0.04	0.05
ATTO 647**N**	AD 647N-141	AD 647N-145	783	683	646	664	150000	0.04	0.03
ATTO 655	AD 655-141	AD 655-145	679	565	663	680	125000	0.24	0.08
ATTO 680	AD 680-141	AD 680-145	677	563	681	698	125000	0.30	0.17
ATTO 700	AD 700-141	AD 700-145	717	603	700	716	125000	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}; $CF_{260} = \varepsilon_{260}/\varepsilon_{max}$; $CF_{280} = \varepsilon_{280}/\varepsilon_{max}$;

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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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.