

## Product Specifications

Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

## Atto 647N

Category	Fluorescent Dyes	
Modification Code	Atto647N-N	
Reference Catalog Number	26-6980	
5 Prime	Υ	
3 Prime	Υ	$\uparrow$
Internal	Υ	5' or 3' Oligo www-NH Atto 647N
Molecular Weight(mw)	843	[26-6980]

This modification is a post synthesis conjugation to a primary amino group thus an additional modification with an amino group is required. A C3, C6 or C12 amino group can be placed at the 5' or for the 3' end a C3 or C7 amino and for internal positions an amino modified base is used, e.g Amino dT C6. **YIELD** NHS based modifications are post synthesis conjugation performed using a primary amino group. The yield is lower as compared to direct automated coupling of modifications that are available as amidites. Approximate yield for various scales are given below.

~2 nmol final yield for 50 nmol scale synthesis.

~5 nmol final yield for 200 nmol scale synthesis.

~16 nmol final yield for 1 umol scale synthesis

Conventional and popular dyes that are derivatives of fluoroscein (FAM, HEX and TET) and Cyanine dye derivatives (Cy3, Cy5, Cy5, Cy7 etc) are commonly used for fluorescently labeling oligos for use as molecular probes for real time PCR, FISH analysis and fragment analysis. For most purposes these provide a good range in wavelength and other optical properties and are available as amidites for direct coupling to oligos using automated chemistry. Other fluorescent dyes are available as N-hydroxysuccinimide (NHS) for conjugation using a primary amine group linked to the oligos. A new series of Atto dyes are now available that are are designed for high sensitivity applications, including single-molecule detection. ATTO 647N belongs to a new generation of fluorescent labels for the red spectral region. Characteristic features of the label are strong absorption, high fluorescence quantum yield, high thermal and photo-stability, and **exceptionally high stability towards atmospheric ozone**. Thus ATTO 647N is highly suitable for single-molecule detection applications and high-resolution microscopy such as SIM, STED etc. Additionally the dye highly qualifies to be applied in flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more.

In common with most ATTO-labels, absorption and fluorescence are independent of pH, at least in the range of pH 2 to 11, used in typical applications. The dye is moderately hydrophilic. ATTO 647N is a cationic dye. After coupling to a substrate the dye carries a net electrical charge of +1. As supplied ATTO 647N consists of a mixture of two isomers with practically identical absorption and fluorescence properties.



The fluorescence is excited most efficiently in the range 625 - 660 nm. A suitable excitation source is the 647 nm line of the Krypton-Ion laser or a diode-laser emitting at 650 nm.

ATTO Dyes are a series of fluorescent labels and dyes manufactured by ATTO-TEC GmbH in Siegen, Germany. The ATTO Dye series covers a spectral range from 390 nm in the UV to 740 nm in the near infrared allowing excitation with most commonly used light sources. The dyes typically are derivatives of coumarins, rhodamines, carbopyronins and oxazines. Compared with other labels especially for the red region of the spectrum, ATTO-labels show excellent photostability and brightness. Atto labels have rigid structures that do not show any cis-trans isomerization. Thus these labels display exceptional intensity with minimal spectral shift on conjugation. The molecules of most common dyes, e.g. cyanines, have a more or less flexible structure. Hence their solutions contain a mixture of several isomers with varying properties. Since the equilibrium between the isomers depends on temperature and other environmental factors, absorption and fluorescence of such dyes are ill-defined. ATTO-dyes have a molecular structure that ensures high rigidity of the chromophore. They do not form equilibria with various isomers, their optical properties are nearly independent of solvent and temperature. ATTO 647N fluoresces twice as strong as Cy5 in aqueous solution. In addition many common fluorescent labels especially cyanine dyes like Cy5 deteriorate even without any irradiation (in the dark), in particular when exposed to small concentrations of ozone present in the laboratory atmosphere. Under identical conditions of ozone exposure the new dyes ATTO 633, ATTO 647N and ATTO 655 last up to 100 times longer than cyanines like Cy5 and Alexa Fluor 647. This is very important in microarray applications, where the dye molecules are located at the surface and thus are in direct contact with the atmosphere.

