

# 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.

### AZDye-647

Category Fluorescent Dyes

Modification Code AZDye-647-N

Reference Catalog Number 26-6479

5 Prime Y

3 Prime Y

Internal Y

Molecular Weight(mw) 970.1

### Click here for a list of fluorophores.

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

### AFDye 647 Abs Maxima is at 655 nm and Emission Maxima is at 680 nm.

Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes. AZDyes are a set of fluorescent dyes that span the visible electromagnetic spectrum, as well as some of the near-IR. The absorbance range is 346-749 nm, and the emission range is 442-775 nm. Generally speaking, AZDyes are brighter, chemically more stable, and less pH-sensitive than other fluorescent dyes commonly used to label oligonucleotides (1). Because they currently only are in the form of NHS esters, oligos first must be synthesized with an Amino Linker modification (either at the ends or internally). The appropriate AZDye is then manually attached to the oligo through the amino group in a separate reaction post-synthesis. The list of currently available dyes includes AZDye 350, -405, -430, -488, -500, -514, -532, -546, -555, -568, -594, -610, -633, -647, -660, -680, -700, -750, with the number indicating the appropriate absorbance wavelength for the particular dye. AZDyes are suitable for a variety of in vitro and in vivo applications. However, for in vivo experiments, users should note that dyes 350/405, being "blue" dyes, require higher-energy excitations than the other. Users of these particular dyes should confirm that the higher-energy required for excitation does not damage the relevant cells or tissues being used in the in vivo experiments.



Near Infrared Fluorophore Spectral Data & Quencher Selection Guide
Fluorophore Name
Excitation Max, nm +/-10
Emission Max, nm +/-10
Extinction Coefficient*
Color**
Quencher
Cy5 650 665 250,000
IRDye 650 NHS 650 665 230,000
AZ647 NHS 655 680 191,800
Cy5.5 684 710 198,000
IRDye 700 NHS 684 710 288,000

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Cy7 NHS 740 773 199,000

IRDye 750 NHS 756 776 260,000

cy7.5 NHS 788 808 223,000

IRDye 800 NHS 795 819 240,000

\* Extinction coefficient at  $\lambda$  (max) in cm-1M-1. \*\* Typical emission color seen through the eyepiece of a conventional fluorescence microscope with appropriate filters. Near-IR region. Human vision is insensitive to light beyond ~650 nm; it is not possible to view near-IR fluorescent dyes.

Click here for a list of fluorophores.

Click here for list of quenchers.

#### References

1. Panchuk-Voloshina, N., Haugland, R.P., Bishop-Stewart, J., Bhalgat, M.K., Millard, P.J., Mao, F., Leung, W-Y., Haugland, R.P. Alexa Dyes, a Series of New Fluorescent Dyes that Yield Exceptionally Bright, Photostable Conjugates. *J. Histochem. Cytochem.* (1999), 47: 1179-1188.

Click here for list of quenchers.

Click here for a list of fluorophores.

Quencher Spectral Data

Quencher

Absorption Max, nm

Quenching Range, nm Dabcyl 453 380-530 BHQ-0 495 430-520 BHQ1 534 480-580 BHQ2 579 550-650 BHQ3 672 620-730

BBQ-650 650 550-750 Click here for complete list of quenchers and details \*\*Black Hole Quencher License Agreement Black Hole Quencher License Agreement. "Black Hole Quencher<sup>®</sup>, BHQ<sup>®</sup>, CAL Fluor<sup>®</sup> and Quasar<sup>®</sup> are registered trademarks of Biosearch Technologies, Inc., Petaluma, California. The BHQ, CAL Fluor and Quasar dye technologies are protected by U.S. and world-wide patents either issued or in application. Compounds incorporating these dyes are made and sold under agreement with Biosearch Technologies, Inc. for end-user's non-commercial research and development use only. Their use in commercial applications is encouraged but requires a separate Commercial Use License granted by Biosearch Technologies, Inc."

#### References

- 1. Didenko, V.V. DNA Probes Using Fluorescence Resonance Energy Transfer (FRET): Designs and Applications. *Biotechniques* (2001), **31**: 1106-1121.
- 2. Livak, K.J., Flood, S.J.A., Marmaro, J., Giusti, W., Deetz, K. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl.* (1995), **4**: 1-6.
- 3. Thelwell, N., Millington, S., Solinas, A., Booth, J., Brown, T. Mode of action and application of Scorpion primers to mutation detection. *Nucleic Acids Res.* (2000), **28**: 3752-3761.
- 4. Tyagi, S., Kramer, F.R. Molecular beacons: probes that fluoresce upon hybridization. Nat. Biotechnol. (1996), 14: 303-308.

