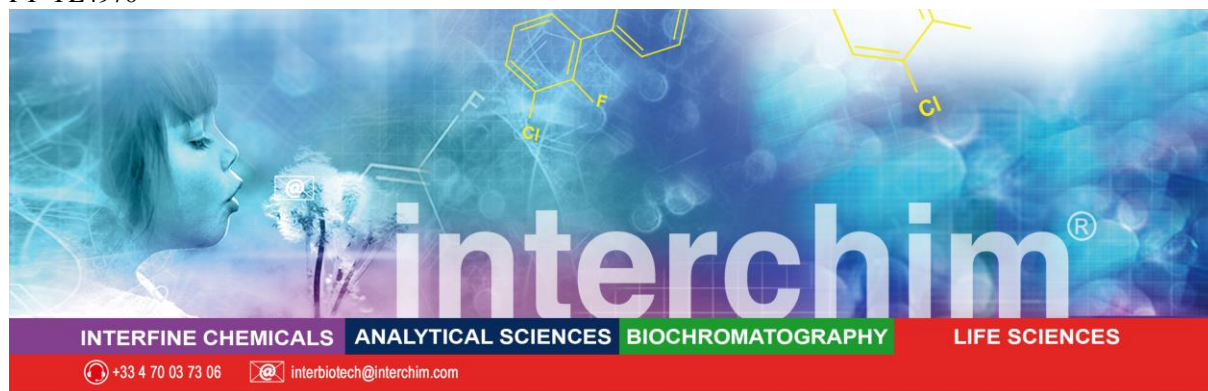


FT-YE4970



Azide FluoProbes® labels

Azide-Fluoprobes® are great fluorescent agents for labeling alkyne-containing molecules

Product Information

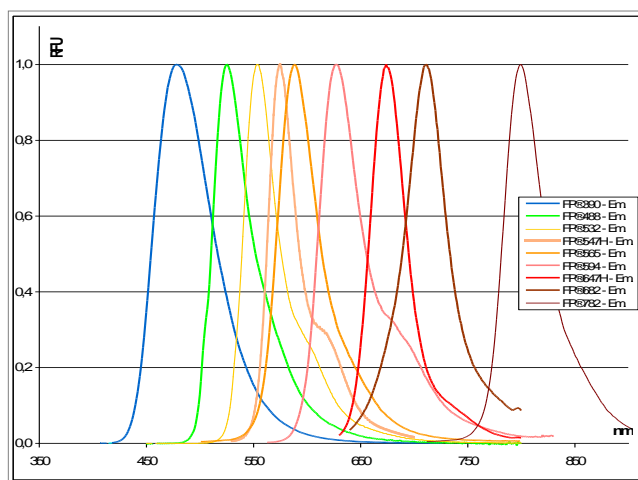
FluoProbes - azide Product cat.number	MW (g·mol ⁻¹)	$\lambda_{exc}/\lambda_{em}$ max. (nm)	mol. abs. (M ⁻¹ cm ⁻¹)	Comment
Fluoprobes® 488 - Azide FP-YE4970, 1 mg	903	494/519	90 000	<ul style="list-style-type: none"> Bright green fluorescence. pH-independent fluorescence between pH 2 and 8 Ultimate photostability, hence minimal fading Compatible with standard filters for FITC, Cy™2 Ideal for confocal microscopy, but suits also any other technique, including microplate readers & FCM.
Fluoprobes® 532A -Azide FP-YE4980, 1mg		532 / 553	115 000	<ul style="list-style-type: none"> excited at 515 - 545 nm (532 nm line Nd:YAG) very bright, with high photostability excellent water solubility, charge of -1 compatible with standard filters for A532
Fluoprobes® 550A -Azide FP-FI2090, 1mg		554 / 576	12 000	<ul style="list-style-type: none"> bright with high thermal and photo-stability moderately hydrophilic, charge of +1 excited at 540 - 565 nm
Fluoprobes® 565A -Azide FP-YE4990, 1mg		563 / 592	120 000	<ul style="list-style-type: none"> excited at 545-575nm dark Red fluorescence with high photo-stability moderately hydrophilic, charge +1 compatible with TR filters
Fluoprobes® 590A- Azide FP-YE5000, 1mg	904	594 / 624	120 000	<ul style="list-style-type: none"> a superior alternative to TR, SR101
Fluoprobes® 633A - Azide FP-YE5010 , 1mg		629 / 657	130 000	<ul style="list-style-type: none"> a superior alternative to A633
Fluoprobes® 647N -Azide FP-YE5020, 1mg	959	644 / 669	150 000	<ul style="list-style-type: none"> Bright red fluorescence Compatible with standard filters for Cy™ 5 High brightness Improved water solubility
Fluoprobes® 655A - Azide FP-YE5030 , 1mg		633 / 684	125 000	<ul style="list-style-type: none"> Highly suitable for single-molecule detection applications and high-resolution microscopy such as PALM, dSTORM, STED etc. Highly qualifies in flow cytometry (FACS), fluorescence in-situ hybridization (FISH) and many more. After coupling to a substrate the dye moiety is electrically neutral.

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FluoProbes - azide Product cat.number	MW (g·mol ⁻¹)	$\lambda_{exc}/\lambda_{em}$ max. (nm)	mol. abs. (M ⁻¹ cm ⁻¹)	Comment
Fluoprobes®-700A - Azide FP-GV3650 , 1mg		700 / 719	120 000	<ul style="list-style-type: none"> • Strong absorption and high fluorescence • Good water solubility • Excellent thermal and photo-stability • Strong electron acceptor. Its fluorescence is efficiently quenched by electron donors like guanine, tryptophan, etc.
Fluoprobes®-725A - Azide FP-LO5630 , 1mg		725 / 752	120 000	<ul style="list-style-type: none"> • Strong absorption and good fluorescence • Excellent thermal and photo-stability • After coupling to a substrate the dye carries a net electrical charge of +1 • pH stable up to 7.4
Fluoprobes®-740A - Azide FP-LO5620 , 1mg		740 / 764	120 000	<ul style="list-style-type: none"> • Strong absorption and good fluorescence • After coupling to a substrate the dye carries a net electrical charge of +1 • pH stable up to 7.4
				•

Storage:

Azide derivatives should be stored at -20°C.. Protect from light.



Other FluoProbes® labels and conjugates

See [related products](#)

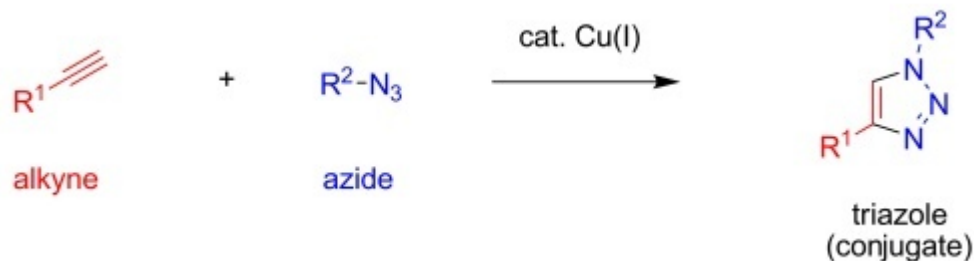
FluoProbes® labels series

Fluoprobes® provides a full range of fluorophores to covers any applications, spanning from 390nm to 800nm.

Fluoprobes® dyes are designed for labeling biomolecules in advanced fluorescent detection techniques. Applications include multiple labeling, FRET, Quenching, polarisation anisotropy fluorescence, and life time resolved fluorescence, with protein as well as with nucleic acids, as well as dyeing materials.

Introduction

Click Chemistry is a reaction between azide and alkyne yielding covalent product - 1,5-disubstituted 1,2,3-triazole. This process is also known as CuAAC - Cu catalyzed alkyne azide cycloaddition.



Click Chemistry is based on copper catalysis. The catalyst is often introduced as Cu-TBTA complex.

Directions for use

Handling and Storage

Fluoprobes® - hydrazide is supplied as dry powder and is stable for at least one year. It is soluble in DMSO

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Coupling carbohydrates or glycoproteins

Click chemistry is a versatile reaction that takes place between two groups: **azide** and **alkyne** (terminal acetylene). It can be used for the synthesis of a variety of conjugates of "partner" biomolecules, once each contains or has been grafted respectively with an azide or an alkyne group. Virtually any biomolecules can be involved, and labeling with small molecules, such as fluorescent dyes, biotin, and other groups can be readily achieved. Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific.

We recommend using the following general protocol for Click chemistry labeling of alkyne-modified oligonucleotides with azides. See [related products](#) for the auxiliary reagents.

Note: The protocol may be adjusted for peptides, proteins and any other molecules including labeling agents. Now, many labels are available already derivatized with azide (an even alkyne) -see also [related products](#) for Azide labels such as biotin and fluoprobes-. Furthermore, the azide and alkyne groups can be incorporated in peptide or oligonucleotides sequence during solid phase synthesis (see alkyne and azide building blocks). Finally, alkyne-modified oligonucleotides or peptides can be ordered on custom synthesis ([please inquire](#)).

Protocol: Click-Chemistry Labeling of Oligonucleotides and DNA

Click chemistry is a versatile reaction that can be used for the synthesis of a variety of conjugates. Virtually any biomolecules can be involved, and labeling with small molecules, such as fluorescent dyes, biotin, and other groups can be readily achieved.

Click chemistry reaction takes place between two components: azide and alkyne (terminal acetylene). Both azido and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific. If there is a need to label an oligonucleotide, alkyne-modified oligonucleotides can be ordered at many of the custom oligo-synthesizing facilities and companies.

- 1- Calculate the volumes of reagents required for Click chemistry labeling using the table below. Prepare the required stock solutions (see Appendix).

Reagent	Final concentration in the mixture	Stock solution concentration
Oligonucleotide, alkyne-modified	Varies (20 – 200 uM)	varies
Azide	1.5 x (oligonucleotide concentration)	10 mM in DMSO
DMSO	50 vol %	-
Ascorbic acid	0.5 mM	5 mM in water
Cu-TBTA complex	0.5 mM	10 mM in 55 vol % DMSO

- 2- Dissolve **alkyne-modified oligonucleotide** or DNA in water in a pressure-tight vial.
- 3- Add **2M triethylammonium acetate buffer, pH 7.0**, to final concentration 0.2 M.
- 4- Add **DMSO**, and vortex.
- 5- Add **azide stock solution** (10 mM in DMSO), and vortex.
- 6- Add the required volume of **5mM Ascorbic Acid Stock solution** to the mixture, and vortex briefly.
- 7- Degass the solution by bubbling inert gas in it for 30 seconds. Nitrogen, argon, or helium can be used.
- 8- Add the required amount of **10 mM Copper (II)-TBTA Stock in 55% DMSO** to the mixture. Flush the vial with inert gas and close the cap.
- 9- Vortex the mixture thoroughly. If significant precipitation of azide is observed, heat the vial for 3 minutes at 80°C, and vortex.
- 10- Keep at room temperature overnight.
- 11- Precipitate the conjugate with acetone (for oligonucleotides) or with ethanol (for DNA). Add at least 4-fold volume of acetone to the mixture (If the volume of the mixture is large, split in several vials). Mix thoroughly and keep at -20°C for 20 minutes.
- 12- Centrifuge at 10000 rpm for 10 minutes.
- 13- Discard the supernatant.
- 14- Wash the pellet with acetone (1 mL), centrifuge at 10000 rpm for 10 minutes.
- 15- Discard the supernatant, dry the pellet, and purify the conjugate by RP-HPLC or PAGE.



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Appendix- Preparation of stock solutions of the reagents used for click-chemistry labeling and conjugation

5 mM Ascorbic Acid Stock

- Preparation Dissolve 18 mg of ascorbic acid in 20 mL of distilled water.
- Storage Ascorbic acid is readily oxidized by air. The solution is stable for one day. Use fresh preparations for Click chemistry.

10 mM Copper (II)-TBTA Stock in 55% DMSO

- Preparation Dissolve 50 mg of copper (II) sulfate pentahydrate in 10 mL of distilled water. Dissolve 116 mg of TBTA ligand in 11 mL of DMSO. Mix two solutions.
- Storage Store at room temperature. The solution is stable for years.

2M Triethylammonium Acetate Buffer, pH 7.0

- Preparation Mix 2.78 mL of triethylamine with 1.14 mL of acetic acid. Add water to 10 mL volume, and adjust pH to 7.0.
- Storage Store at room temperature. The solution is stable for years.

Related products and documents

- Sulfo-NHS-Acetate #[UP69380A](#)
- SFB #[M11771](#)
- EDAC #[52005A](#)
- Reducers: DTT #[UP284250](#), TCEP #[UP242214](#)
- Desalting: [UptiSpin](#) filters; Gelfiltration G-25 columns # [84874](#); [CelluSep](#) dialysis tubings
- PBS buffer #[UP68723A](#)
- 5-DBCO-dUTP, [JO2460](#)

- FluoProbes labeling agents: See [selected most popular and remarkable labels](#), [BioSciences catalogue](#) p.[B56](#). Other derivatives are available, incl. amino-, carboxy-, [Succinimidyl-](#), [Hydrazide-](#), [Azide](#), (strept)avidin, secondary antibodies, some specific probes such as Annexin, Phalloidin, ... or any other on custom labeling. FluoProbes® [Protein labeling Kits](#)
- Fluorescent labeling of proteins to analyze in electrophoresis (2D-gel PAGE): [NT-2D](#).

Ordering information

Catalogue size quantities and prices may be found at <http://www.fluoprobes>.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes / Interchim; Hotline : +33(0)4 70 03 73 06

Disclaimer : Materials from FluoProbes® are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use.

