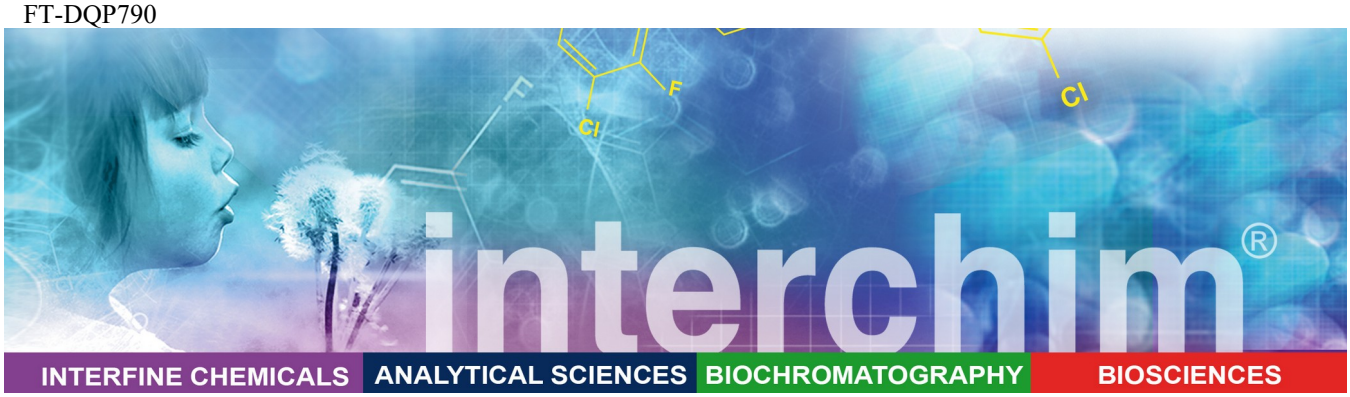


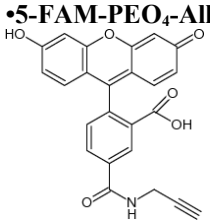
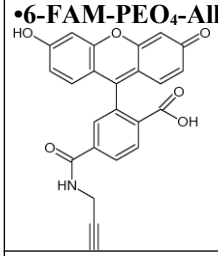
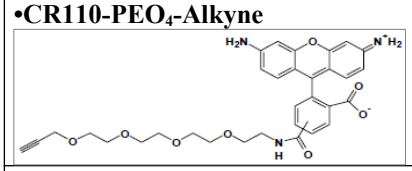
Classic fluorescent dyes conjugated to Alkyne and DBCO – for Click chemistry

Products Description

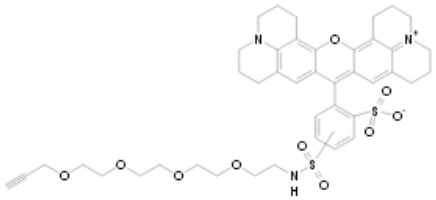
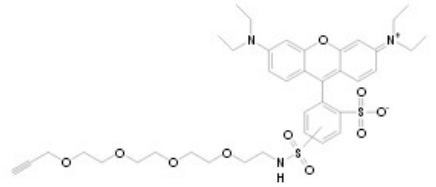
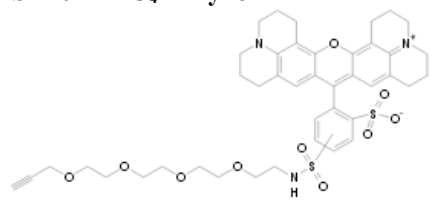
Alkyne and DBCO activated conventional fluorescent dyes, for labeling azide-containing biomolecules by via Cu(I)-catalyzed Click Chemistry (for Alkynes) or via Copper-free Click Chemistry (for DBCO).

FAM, CR110, CR6G, TAMRA, SRB, SR101(TR), and CYanine3/5/5.5/7 dyes are classic fluorescent dyes used in a variety of detection techniques. They are provided activated by **DBCO** and **alkyne** functional groups to perform easy conjugation of biomolecules (proteins, peptides, amino-modified DNAs and oligos).
- PEO spacer confers better solubility.
- DBCO functional group allows conjugation to any biomolecule derivated by Azide via SPAAC reaction, without requiring a catalyzer like standard Click Chemistry that requires Cu(I) to catalyze alkyne / azide reaction. Both DBCO and alkyne groups are nearly never encountered in natural biomolecules. Hence, the reaction is highly bioorthogonal and specific.

- **Alkynes for conjugation to Azide modified biomolecules via Cu(I)-Click Chemistry :**

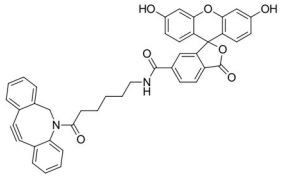
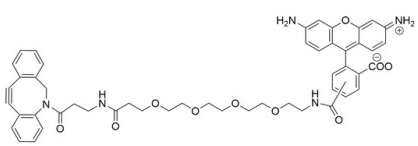
Fluorescent dye – conjugate	Cat.number	MW	$\lambda_{Abs./Em.}$ (nm)	EC	QY	Store
•5-FAM-PEO$_4$-Alkyne 	FP-8K6700, 5mg CAS: 510758-19-7; 5--Fluorescein-Alkyne Abs/Em = 490/513 nm; EC: 80 000; Yellow to orange solid.	413.38	490/513nm	80 000		(M)
•6-FAM-PEO$_4$-Alkyne 	FP-8K6710, 5mg CAS:478801-49-9; 6-Fluorescein-Alkyne	413.38	490/513nm	80 000		(M)
•CR110-Alkyne	Inquire, #TA106 (M); Soluble in DMSO, DMF	588.63	488/523nm	74 000		(M)
•CR110-PEO$_4$-Alkyne 	FP-DQP791, 5mg 5/6-Carboxyrhodamine 110-PEG$_4$-Alkyne, Fluor488-PEG$_4$-Alkyne; Acetylene-Fluor 488 MW: 588.62 (M); Soluble in DMSO, DMF, MeOH Abs/Em = 501/525 nm; EC: 74-85000	587.62	501/525nm	74 000		(M)
•TAMRA-Alkyne	Inquire, #1255	643.73	553/575nm	89 000		(M)

FT-DQP790

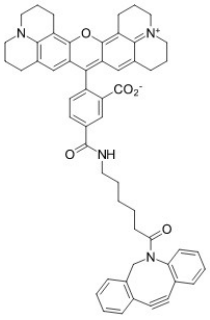
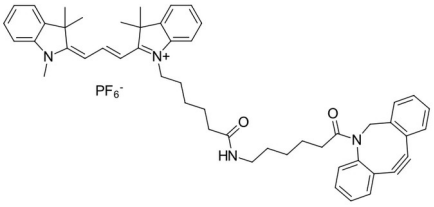
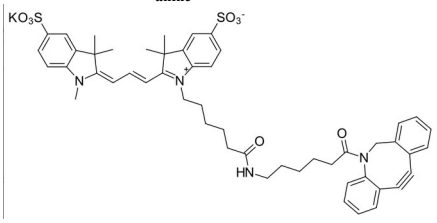
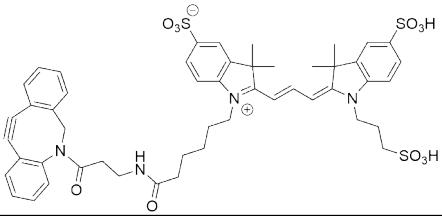
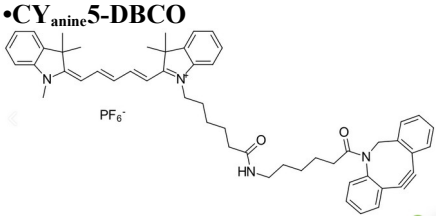
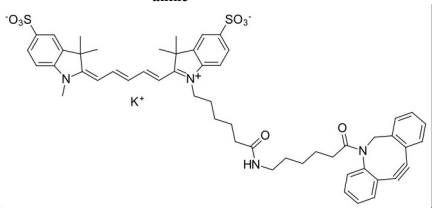
Fluorescent dye – conjugate	Cat.number	MW	$\lambda_{Abs./Em.}$ (nm)	EC	QY	Store
	(M); Soluble in DMSO, DMF					
•TAMRA-PEO$_4$-Alkyne 	FP-DQP811, 5mg	643.73	546/565nm	92 000		(M)
	5/6-Carboxytetramethylrhodamine-PEG$_4$-Alkyne, Fluor545-PEG$_4$-Alkyne MW: 643.73 (M); Soluble in DMSO, DMF, MeOH Abs/Em = 546/565 nm; EC: 92000					
•SRB-Alkyne	Inquire		568/584nm			(M)
	(M); Soluble in DMSO, DMF					
•SRB-PEO$_4$-Alkyne 	FP-DQP821, 5mg	778	568/584nm	106 000		(M)
	5/6-SulfoRhodamineB-PEG$_4$-Alkyne , Fluor568-PEG$_4$-Alkyne, Acetylene-SulfoRhodamineB, (also known as Lissamine™rhodamine B, -Fluor568) MW: 778 (M); Soluble in DMSO, DMF, MeOH Abs/Em = 568/584 nm; EC: 91-106000					
•SR101-PEO$_4$-Alkyne 	FP-DQP831, 5mg		584/603nm	110 000		(M)
	5/6-SulfoRhodamine101-PEG$_4$-Alkyne , TR-PEG$_4$-Alkyne, Acetylene-Fluor 585, Fluor585-PEG$_4$-Alkyne MW: 1112.31(M); Soluble in DMSO, DMF; Abs/Em = 584/603 nm; EC: 97-110000					
•CY$_{anine}$-Alkyne see technical sheet FT-1A6320:	Sulfo-CY$_{anine}$3-Alkyne #FP-1A6320			555 / 570nm		
	Mono-Sulfo-CY$_{anine}$3-Alkyne #FP-1C4620 Di-SulfoCy3-Alkyne #FP-LQV030, 1C4630, 0B8390 Tri-Sulfo-CY$_{anine}$3-Alkyne #FP-1C8831					
	Cy5-alkyne #FP-OO5590			646 / 662nm		
	Di-Sulfo-Cy5-alkyne #FP-LQV090, 1C4640, SJI060 Tri-Sulfo-CY$_{anine}$5-Alkyne #FP-1C8841					
	Cy5.5-alkyne #FP-SJH910			673 / 707nm		
	Tri-Sulfo-CY$_{anine}$5.5-Alkyne #FP-MRV061 Tetra-Sulfo-Cy5.5-alkyne #FP-LQV320					
	Cy7-alkyne #FP-FP-WZE120			749 / 776nm		
	Di-Sulfo-Cy7-alkyne #FP-LQV250 Tri-Sulfo-CY$_{anine}$7-Alkyne #FP-1I1221					

§: Inquire for other sizes (1mg size(0), 25mg(2); bulk)

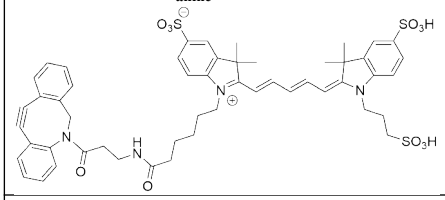
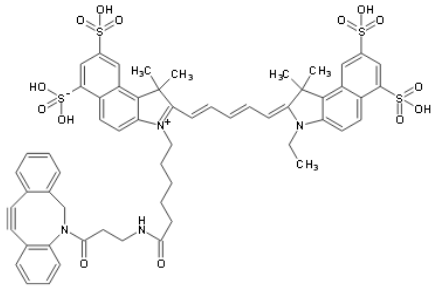
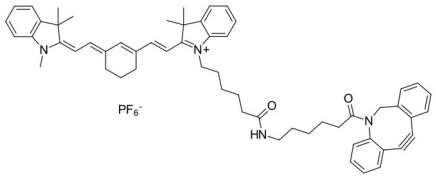
• DBCO for conjugation to Azide modified biomolecules via Copper-free Click Chemistry:

	Cat.number	MW	$\lambda_{Abs./Em.}$ (nm)	EC	QY	(Store)
•FAM-DBCO, 6-isomer 	FP-B35T72, 1mg	676.71	494/520nm	75 000	0.9	
	Soluble in DMF, DMSO					
•CR110-PEO$_4$-DBCO 	FP-1C8681, 5mg	879.97	501/525nm	74 000		(M)
	5/6-Carboxytetramethylrhodamine-PEG$_4$-Dibenzylcyclooctyne, Dibenzylcyclooctyne-PEG$_4$-CarboxyMethylRhodamine-110, Also known as -Fluor 488; MW: 879.97 (M) Abs/Em = 501/525 nm; QY: 74000 (in MeOH) ; CF_{260}: 0.24, CF_{280}: 0.19 Soluble in DMSO, DMF					
	On inquire: CR6G-DBCO (MW: 632.67) n°FP-DQP850					

FT-DQP790

	Cat.number	MW	$\lambda_{Abs./Em.}$ (nm)	EC	QY	(Store)
•TAMRA-PEO$_4$-DBCO	FP-1C8691, 5mg	~1000	545/565nm	92 000		(M)
	5/6-Carboxytetramethylrhodamine-PEG$_4$-Dibenzylcyclooctyne, Dibenzylcyclooctyne-PEG$_4$-TetraMethymRhodamine,, Fluo545-PEG$_4$-DBCO MW:~1000 (M) Abs/Em = 546/565 nm; QY: 80 000 (in MeOH) ; CF$_{260}$: 0.30, CF$_{280}$: 0.27 Soluble in DMSO, DMF – better solubility thanks to the PEO spacer nature. On inquire: TAMRA-DBCO (MW: 688.78) n°FP-DQP881					
•ROX-DBCO, 5-isomer	FP-B8J721, 5mg	835.02	570/591nm	93000		
	Soluble in DMF, DMSO					
•CY$_{anine}$ - DBCO	CyDyes dyes, activated by –Azide : see the tech sheet FT-xxxx-1A6320					
•CY$_{anine}$3-DBCO	FP-WXS881, 5mg					
	MW:793.48 Abs/Em = 555/570nm; EC: 150 000; QY: 0.31; CF$_{260}$: 0.04, CF$_{280}$: 0.09 Soluble in DMF, DMSO, DCM, alcohols See technical sheet FT-AWHFX0					
•Di-Sulfo-CY$_{anine}$3-DBCO	FP-B35TA1, 5mg					
	MW :955.23(916.4 added mass) Abs/Em = 548/563 nm; EC: 162 000; QY:0.1; CF$_{260}$:0.03 ; CF$_{280}$:0.06 Apparence: red solid. Soluble in water, DMF, DMSO					
•Tri-Sulfo-CY$_{anine}$3-DBCO	FP-1C8721, 5mg					
	CAS: 1782950-79-1 Soluble in Water, DMSO, DMF Alternative to AF555, A555, CF555, DL549					
•CY$_{anine}$5-DBCO	FP-AWHFX1, 1mg	929.03	646/662	250000	0.2	
	MW :929.03(928.4 added mass) Abs/Em = 624/662 nm; EC: 250 000; QY:0.2; CF$_{260}$:0.03; CF$_{280}$:0.04 Soluble in DMF, DMSO, chlorinated organic solvents See technical sheet FT-AWHFX0					
•Di-Sulfo-CY$_{anine}$5-DBCO	FP-B431I1, 5mg					
	MW :981.27() Abs/Em = 646/662 nm; EC: 271 000; QY:0.28; CF$_{260}$:0.04 ; CF$_{280}$:0.04 Apparence: dark colored solid. Soluble in water, DMF, DMSO					

FT-DQP790

	Cat.number	MW	$\lambda_{Abs./Em.}$ (nm)	EC	QY	(Store)
•Tri-Sulfo-CY$_{anine}$5-DBCO 	FP-1C8731, 5mg CAS 1564286-24-3 Soluble in Water, DMSO, DMF Alternative to AF647, A647, CF647, DL649					
•CY$_{anine}$5.5-DBCO	FP-B35T31, 5mg MW: 1029.44 (882.5 added mass) Abs/Em =684 710/nm; EC: 209 000; QY: 0.2; CF_{260}: 0.07, CF_{280}: 0.03 See technical sheet FT-AWHFX0					
•Tetra-Sulfo-CY$_{anine}$5.5-DBCO 	FP-MRV031, 5mg MW:1478.94(TEA alt), 175.37(protonated) Soluble in Water, DMSO, DMF, MeOH Abs/Em = 678/694 nm, EC: 190000; CF_{260}: 0.05, CF_{280}: 0.18					
•CY$_{anine}$7-DBCO 	FP-AWHG01, 1mg	885.62	750/773	199000	0.3	
	MW: 885.62 (849.5 added mass) Abs/Em = 750/773nm; EC: 199 000; QY: 0.3; CF_{260}: 0.022, CF_{280}: 0.025 See technical sheet FT-AWHFX0					
•Tetra-Sulfo-CY$_{anine}$7-DBCO	FP-MRV041, 5mg					
	Soluble in Water, DMSO, DMF Alternative to AF750, 750RD, CF750, DL750					
	See also **DBCO - PEGx - CYanines, MW 1-40KDa** (FT-1Q7081) High molecular mass, purified compound (polydisperse)					

§ Inquire for other size (1mg size(0), 5mg(1), 10mg(2), 25mg(3); bulk)

Technical information

Information on fluorescent labels:

● **CR110** (5/6-Carboxyrhodamine 110) can be excited by the 488nm spectral line of the argon-ion laser. Fluorescence is insensitive to pH4-9 – more stable than A488, which is readily degraded under alkaline condition Photostability is excellent (better than FITC and A488)

● **CR6G** (5/6-Carboxyrhodamine 6G-PEG_4-Alkyne) can be excited by the 514nm spectral line of the argon-ion laser. Fluorescence is insensitive to pH4-9 and has excellent photostability.

● **TAMRA** (5/6-Carboxytetramethylrhodamine) can be excited by the 546nm spectral line of the mercury-arc lamps, and also the 543nm line of the Ar-Kr mixed gas laser. Fluorescence uis insensitive to pH4-9. Photostability is excellent

● **SRB** (5/6-SulfoRhodamineB), with max absorption at 563 nm, is readily excited by the 568 nm spectral line of the Ar – Kr mixed gas laser. tend to exhibit a higher fluorescence intensity than nonsulfonated rhodamine conjugates. The fluorescence is pH insensitive between 4 and 9, and exhibits excellent photostability.

● **SR101** (5/6-SulfoRhodamine101)
Unlike other rhodamine based probes, SR101 exhibits very little spectral overlap with fluorescein. With peak absorption at 584 nm. It is particularly well suited for excitation by the 568 nm spectral line of the Ar – Kr

mixed gas laser commonly used in many confocal laser-scanning microscopes or the 594 nm spectral line of the orange He-Ne laser.
The fluorescence quantum yield of the Dibenzylcyclooctyne-Fluor 585 probe is higher than that of tetramethylrhodamine or SRB. Usually, conjugates exhibit higher fluorescence intensity than conjugates of other rhodamine based probes

● **CY$_{anine}$3** (Cy3) can replace Fluoresceins, A546 and DyLight 549&550 dyes. See superior FluoProbes547H.
Cy3 is one of the most broadly used fluorophore which can be detected by various fluorometers, imagers, and microscopes with a wavelength range of 530-555 nm. Due to inherently high extinction coefficient, this dye is also easily detected by naked eye on gels, and in solution.

● **CY$_{anine}$5** (Cy5) can replace TMR, A647, DyLight649&650, CF647, C645A, PF647. See superior FP647H.
Cy5 is excited maximally at 650 nm to about 98% of maximum with a krypton/argon laser (647 nm line) or to about 63% of maximum with a helium/neon laser (633 nm line). It fluoresce maximally at 670 nm, that has a lower autofluorescence of biological specimens than shorter wavelenghts. Cy5 can be used with a variety of other fluorophores for multiple labeling due to a wide separation of its emission from that of shorter wavelength-emitting fluorophores. However, emission cannot be seen well by eye, and Cy5 cannot be excited optimally with a mercury lamp. Therefore, this dye is not recommended for use with conventional epifluorescence microscopes. It is most commonly visualized with a confocal microscope equipped with an appropriate laser for excitation and a far-red detector. Cy5 and FP547H is less expensive and equally bright alternative to Allophycocyanin conjugates for flow cytometry.

Information on functional groups (Alkyne, DBCO)

Alkyne and DBCO both react with Azide groups (Click reactions).
●**Alkyne** (Acetylene moiety) readily couples Azide modified biomolecules (standard Click reaction using Cu catalyzer).
●**DBCO** (Dibenzylcyclooctyne) readily couples Alkyne modified molecules (SPAAC Click reactions without the need of Cu catalyzer).

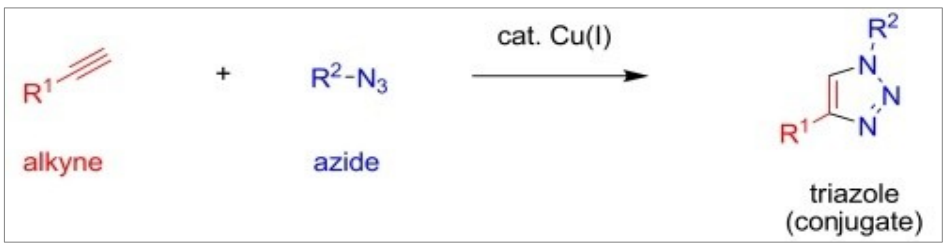
Click Chemistry

Among the vast variety of organic reactions, Click Chemistry has been selected as a conjugation chemistry reaction because of several advantages:

►**It is very selective.** Click Chemistry reaction takes place only between azide and alkyne components. It is does not interfere with most any other organic groups present in DNA and proteins being labeled, such as amino and carboxy groups.
►**There are no azides and alkynes in native biomolecules.** These groups should be specially introduced into DNA and proteins. Alkyne-containing DNA can be prepared with alkyne phosphoramidite+ during standard oligo synthesis. Proteins labeled with azide and alkyne can be made using azide activated ester+ and alkyne activated ester+.
►**Click Chemistry takes place in water.** Aqueous DMSO, DMF, acetonitrile, alcohols, or pure water and buffers can be used for the reaction. The reaction is biocompatible and can take place in living cells.
►**Reaction is quick and quantitative.** Click Chemistry is a tool that allows preparation of nanomoles of conjugates in diluted solutions.
►**The reaction is pH-insensitive.** Unlike reaction of NHS esters with amines, and some other conjugation chemistries, there is no need to control pH in reaction mixture. There is no need to add any special buffer, acid or base - Click Chemistry works well in pH interval of 4-11.
Click Chemistry thus became a tool for universal modification of DNA, proteins, conjugate preparation, and fluorescent labeling. This is where our reagents come to help: you can perform easy preparation of conjugates in your lab.

- **Click Chemistry reaction – CuAAC**

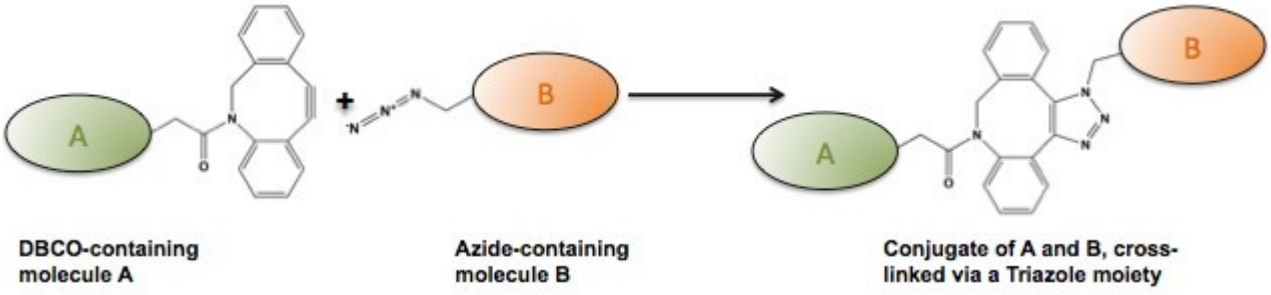
Click Chemistry is a reaction between azide and alkyne yielding covalent product - 1,5-disubstituted 1,2,3-triazole. This process is also known as CuAAC - Cu catalyzed alkyne azide cycloaddition.



The catalyst is often introduced as Cu-TBTA (**Copper(II)-TBTA complex** FY2780).

- **Click Chemistry reaction – SPAAC**

The novel **Copper-free Click Chemistry** is based on the reaction of a diarylcyclooctyne (DBCO) moiety with an azide-labeled reaction partner, known as **s**train-**p**romoted **a**lkyne **a**zide **c**ycloaddition (SPAAC). This new "Click reaction", unlike conventional Click Chemistry, is **very fast** at room temperature and **does not require a cytotoxic Cu(I) catalyst (**that is toxic to most organisms) and thus, prevents its use in many biological systems. Diarylcyclooctynes are thermostable with very narrow and specific reactivity toward azides, resulting in **almost quantitative yields of stable triazoles**.



Protocols

A - General protocols for standard click chemistry (Cu-catalyzed)

See the technical sheet FT-FY2780 for more information on Click Chemistry and protocols.
> Click Chemistry Labeling of Oligonucleotides and DNA

B - Assay Procedure for Live Cell Labeling using DBCO-PEO_4-TAMRA

• Grow mammalian cells in an appropriate medium with an azide-derivatized metabolite (e.g. ManNAz) at 37°C in 5 % CO_2.

• Wash the cells two times with D-PBS containing 1 % FBS.

• Prepare a 5 mM stock solution of DBCO-PEO_4-TAMRA in a water-miscible solvent such as DMSO or DMF by adding 0.427 ml of solvent to 2 mg vial or 1.07 ml to a 5 mg vial and vortex to dissolve all solid.

• Label the azide-modified cells at room temperature in the dark for 30 - 60 min with 5 to 30µM of DBCO-PEG4-TAMRA in D-PBS containing 1 % FBS.

• Wash the cells four times with D-PBS containing 1 % FBS.

• Fix the cells with 4 % formaldehyde in D-PBS for 20 min at room temperature.

• Wash the cells with D-PBS.

• Optional step: Counterstain the cells for 15 min at room temperature with Hoechst 33342 in D-PBS.

• Wash the cells two times with D-PBS.

• Image the cells.

Lysing Cells

Note: Do not use DTT, TCEP or β-mercaptoethanol, because they will reduce the azide.

•Prepare lysis buffer (100 mM Tris buffer pH 8.0 containing 1 % (w/v) SDS).

• Optional step: Add protease and phosphatase inhibitors to the lysis buffer.

• Suspend cells in the lysis buffer (50µl lysis buffer per 10^6 cells) and heat to 75°C. If using a 6-well plate you need 500µl lysis buffer per 100 mm dish and 200µl lysis buffer per well.

• Sonicate the lysate briefly to shear DNA and reduce the viscosity of the solution.

• Vortex the lysate for 5 min.

• Centrifuge the cell lysate at 16,000 g at 4°C for 10 min.

• Transfer the supernatant to a clean tube and determine the protein concentration if required. Ideally, the protein concentration should be 1-2 mg/ml.

• Prepare 1 M solution of iodoacetamide by adding 3 ml of DMSO to IAA labeled vial (provided with a lysis labeling kit).

•Block cysteine thiols in lysate by addition of iodoacetamide stock solution to a final concentration of 15mM, agitate mildly for 30 min.

• Prepare a 5 mM stock solution of DBCO-PEG4-TAMRA by adding 0.427 ml of DBCO to 2 mg vial or 1.06 ml to 5 mg vial.

• Label the cell lysate by addition of DBCO-PEG4-TAMRA to a final concentration of 20 M. Protect from light and agitate mildly for 30 min at room temperature.

• Prepare a 50 mM stock solution of stop buffer by adding 3 ml of water to Stop Reagent labeled vial (provided with a lysis labeling kit).

• Stop reaction by addition of stop buffer to a final concentration of 100M, agitate briefly for 20 min.

• Load 10 of protein on 12 % Tris-Tricine SDS-PAGE gel.

References

Ngo et al. (2012) State-selective metabolic labeling of cellular proteins. ACS Chemical Biology. 7(8) :1326.

Rubino et al. (2012) Chemoselective Modification of Viral Surfaces via Bioorthogonal Click Chemistry. J. Vis. Exp. 66 :4246.

Yao et al. (2012) Fluorophore Targeting to Cellular Proteins via Enzyme-Mediated Azide Ligation and Strain-Promoted Cycloaddition. J. Am. Chem. Soc. 134 :4246.

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