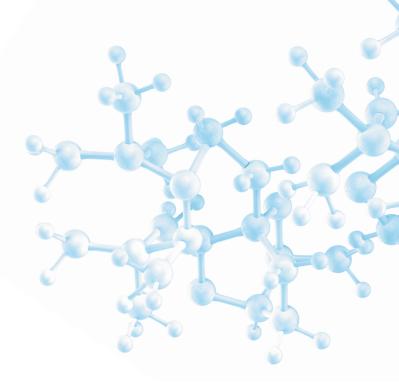




# **Click Chemistry Toolbox**

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- Fluorescent Probes for Cu-free Click Chemistry
- Fluorogenic Azides Probes
- Azide Plus Next Generatin Azide Probes
- Iso-TaG Reagents and Kits
- Cleavable Click Chemistry Biotin Probes
- Click Chemistry Enrichment Kits and Media





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# **Copper-Catalyzed Click Reaction**

$$R_1 \longrightarrow R_2 \longrightarrow R_1 \longrightarrow R_1 \longrightarrow R_2$$
Azide Alkyne Triazole

**Figure 1** Schematic representation of copper-catalyzed click reaction.

Click reactions are defined more broadly as those that meet the necessary criteria of being selective, high yielding, wide in scope and having good reaction kinetics. These reactions possess extreme selectivity and biocompatibility, such that their participating reagents can form covalent bonds within richly functionalized biological systems—in some cases, living organisms.

Among many click reactions described up to date, the most widely used reaction is the Huisgen 1,3-dipolar cycloaddition of alkynes to azides to form 1,4-disubsituted-1,2,3-triazoles (**Figure 1**). The copper(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC) is mild and very efficient, requiring no protecting groups, and requiring no purification in many cases. The azide and alkyne functional groups are largely inert towards biological molecules and aqueous environments. Unlike other labels, the azide-and alkyne-tags are small enough that tagged biomolecules (e.g., azide- or alkyne-containing sugars, amino acids and nucleotides) are acceptable substrates for the enzymes that incorporate these building blocks into biopolymers such as proteins, DNA and RNA. This unique property paved the way for a very powerful, innovative and simple two-step labeling procedure. In the first step, an azide- or alkyne-containing biomolecule is actively incorporated into the protein. The second step, the detection step, uses the chemoselective ligation or "click" reaction between an azide and an alkyne. In the click reaction, the modified protein is detected with a corresponding azide- or alkyne-containing dye or hapten.

This powerful two-step procedure enables a large number of applications, such as detection of global RNA/DNA synthesis temporally and spatially in cells and tissues; detection and characterization of newly synthesized proteins; changes in spatial or temporal protein expression patterns; protein degradation resulting from disease, drug treatments, or environmental changes; visualization and characterization of various post-translational modifications (e.g. glycosylation, acylation, phosphorylation); and imaging bacterial cell wall biosynthesis.

## Click-&-Go® Click Chemistry Reaction Buffer Kit

The Click-&-Go® Click Chemistry Reaction Buffer Kit provides researchers — who have biomolecules labeled with an azide or alkyne and the corresponding click detection reagent — with all of the necessary reagents to perform a copper-catalyzed ligation reaction. Sufficient materials are provided to perform up to 25 copper-catalyzed click reactions for subsequent analysis by gel electrophoresis, western blot or mass spectrometry.



Product #	Description	Pkg. Size
CCT-1001	Click-&-Go® Click Chemistry Reaction Buffer Kit	1 kit

#### Click-&-Go® Cell Reaction Buffer Kit

The Click-&-Go® Cell Reaction Buffer Kit provides researchers with everything required to perform a click reaction on cells tagged with an azide or alkyne with the corresponding click detection reagent for subsequent downstream analysis.

The performance and components of this kit are identical to Click-iT® Protein Reaction Buffer Kit from Thermo Fisher Scientific (Cat# C10269).

Product #	Description	Pkg. Size
CCT-1263	Click-&-Go® Cell Reaction Buffer Kit	1 kit

## Click-&-Go® Protein Reaction Buffer Kit

The Click-&-Go® Protein Reaction Buffer Kit provides researchers with everything required to perform a click reaction on azide or alkyne tagged proteins with the corresponding click detection reagent for subsequent downstream analysis.

The performance and components of this kit are identical to Click-iT® Protein Reaction Buffer Kit from Thermo Fisher Scientific (Cat# C10276).

Product#	Description	Pkg. Size
CCT-1262	Click-&-Go® Protein Reaction Buffer Kit	1 kit

# Click Chemistry Auxiliary Reagents

THPTA			Catalog#	Unit
CAS: MW: Solubility: Description:	760952-88-3 434.50 Water, DMSO, DMF Water-soluble chelating agent	HO N=N OH	CCT-1010-100 CCT-1010-500 CCT-1010-1000 CCT-1010-5G	100 mg 500 mg 1000 mg 5 g
ВТТАА			Catalog#	Unit
CAS: MW: Solubility: Description:	1334179-85-9 430.52 Water, DMSO, DMF Water-soluble chelating agent	N-N N-N N-N COOH	CCT-1236-100 CCT-1236-500 CCT-1236-1000	100 mg 500 mg 1000 mg
BTTES			Catalog#	Unit
CAS: MW: Solubility: Description:	n/a 494.62 Water, DMSO, DMF Water-soluble chelating agent	N N N N N N N N N N N N N N N N N N N	CCT-1237-100 CCT-1237-500 CCT-1237-1000	100 mg 500 mg 1000 mg
ВТТР			Catalog#	Unit
CAS: MW: Solubility: Description:	n/a 430.56 DMSO, DMF, MeOH Water-soluble chelating agent	N-N N N N N N N N N N N N N N N N N N N	CCT-1414-100 CCT-1414-500 CCT-1414-1000	100 mg 500 mg 1000 mg
ТВТА			Catalog#	Unit
CAS: MW: Solubility: Description:	510758-28-8 530.62 Water, DMSO, DMF Chelating agent		CCT-1061-100 CCT-1061-500 CCT-1061-1000	100 mg 500 mg 1000 mg



# Click-&-Go® Plus Imaging Kits

The Click-&-Go® Plus Imaging Kit is a general purpose imaging kit that is designed to perform a high sensitivity imaging of moderate-to-low abundance alkyne-containing biomolecules. The labeling kit utilizes the latest generation of copper-chelating azide capable of forming strong, active copper complexes that react almost instantaneously with alkynes under diluted conditions.

Each Click-&-Go® Plus Imaging Kit includes the fluorescent azide plus probe and all of the reagents required to create a reaction cocktail.

Product #	Description	Pkg. Size
CCT-1313	Click-&-Go® Plus 405 Imaging Kit	1 kit
CCT-1314	Click-&-Go® Plus 488 Imaging Kit	1 kit
CCT-1315	Click-&-Go® Plus 532 Imaging Kit	1 kit
CCT-1316	Click-&-Go® Plus 546 Imaging Kit	1 kit
CCT-1317	Click-&-Go® Plus 555 Imaging Kit	1 kit
CCT-1318	Click-&-Go® Plus 568 Imaging Kit	1 kit
CCT-1319	Click-&-Go® Plus 594 Imaging Kit	1 kit
CCT-1320	Click-&-Go® Plus 647 Imaging Kit	1 kit



# Click-&-Go® Plus OPP Protein Synthesis Assay Kits

Click-&-Go® Plus OPP Protein Synthesis Assay Kits enable fast, sensitive, and non-radioactive detection of protein synthesis using fluorescence microscopy or high-content imaging. O-propargyl-puromycin (OPP), an analog of puromycin that contains a terminal alkyne group, enters the acceptor site of ribosomes and incorporates into nascent polypeptide chains. OPP is not an amino acid analog, thus, OPP can be added directly to cells in complete media (i.e., methionine-containing) or used to detect in vivo protein synthesis. OPP that is incorporated into newly translated proteins is detected with fluorescent azides though a fast, highly-specific, and mild click reaction.

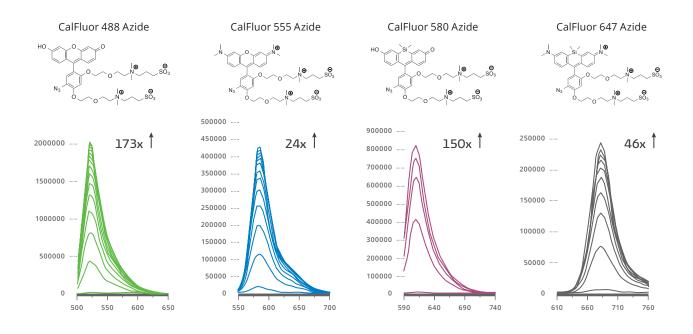
Product#	Description	Pkg. Size
CCT-1492	Click-&-Go® Plus 405 OPP Protein Synthesis Assay Kit	1 kit
CCT-1493	Click-&-Go® Plus 488 OPP Protein Synthesis Assay Kit	1 kit
CCT-1494	Click-&-Go® Plus 555 OPP Protein Synthesis Assay Kit	1 kit
CCT-1495	Click-&-Go® Plus 594 OPP Protein Synthesis Assay Kit	1 kit
CCT-1496	Click-&-Go® Plus 647 OPP Protein Synthesis Assay Kit	1 kit

yl-puromycin (OPP)	N	Catalog#	Unit
1416561-90-4	HO N N	CCT-1407-5	5 mg
495.54	O NH OH	CCT-1407-25	25 mg
DMSO, DMF, water (pH adjusted to 5.0)	1	CCT-1407-100	100 mg
	1416561-90-4 495.54	1416561-90-4 495.54	1416561-90-4 CCT-1407-5 495.54 CCT-1407-25 DMSO, DMF, water (pH adjusted to 5.0) CCT-1407-100

# **CalFluor Azide Probes**

A major shortcoming of the visualization of alkyne-tagged biomolecules with fluorescent azide probes through CuAAC reactions is the need to remove unreacted fluorescent probes. This is particularly problematic when imaging the intracellular environment, tissues of living organisms, or visualizing biomolecules in vivo. The difficulty of removing all unreacted fluorescent probes is also one of the major contributors to background signal and non-specific binding.

To overcome this shortcoming, the Carolyn Bertozzi group has designed fluorogenic azide probes that are activated by Cucatalyzed or metal-free click chemistry. These azide probes are not fluorescent until they react with alkynes. Termed the CalFluors, these probes possess emission maxima that range from green to far-red wavelengths, and enable sensitive biomolecule detection under no-wash conditions. A number of reports showed that CalFluor probes are an indispensable tool for sensitive visualization of metabolically labeled molecules (glycans, DNA, RNA, and proteins) in cells, developing zebrafish, and mouse brain tissue slices under no-wash conditions.



Description	Ex/Em	Emission Color	Pkg. Size	Product #
CalFluor 488 Azide	500/521		1 mg	CCT-1369-1
Carridor 400 Azide		Green	5 mg	CCT-1369-5
CalFluor 555 Azide	561/583	D - 4	1 mg	CCT-1370-1
Calridor 555 Azide	301/363	Red	5 mg	CCT-1370-5
CalFluor 580 Azide	591/609	Red	1 mg	CCT-1371 - 1
Calridol 300 Azide	391/009	Reu	5 mg	CCT-1371 - 5
CalFluor 647 Azide	657/674	Near IR	1 mg	CCT-1372 - 1
Cairiuui 647 Azide	05//0/4		5 mg	CCT-1372-5

#### **Selected References:**

Shieh P., et al. (2015). CalFluors: A Universal Motif for Fluorogenic Azide Probes across the Visible Spectrum J. Am. Chem. Soc., 137: 7145 – 51. Pawlak, J. B., et al. (2016). The Optimization of Bioorthogonal Epitope Ligation within MHC-I Complexes. ACS Chem. Biol., 11: 3172 – 8.

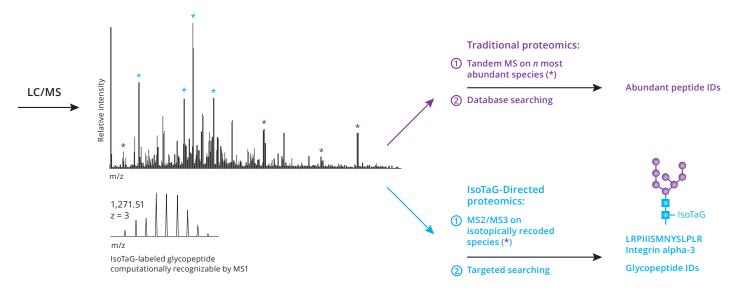
CalFluor Azide Probes are covered by U.S. Patent No.: 9,410,958.

This product may be used for research purposes only. It is not licensed for resale and may only be used by the buyer. This product may not be used and is not licensed for clinical assays, where the results of such assays are provided as a diagnostic service. If a diagnostic or therapeutic use is anticipated, then a license must be requested from the University of California. The availability of such diagnostic and therapeutic use license(s) cannot be guaranteed from the University of California.

# IsoTaG Reagents and Kits

A popular strategy for protein identification is the bottom-up shotgun proteomics approach. In this method, a mixture of proteins is subjected to proteolytic digestion, the resulting peptides are separated by LC and detected by MS, and their parent proteins are inferred from the assigned peptide sequences.

To convert MS data acquired from proteolytic digests into protein identifications, tandem MS can be used to obtain sequence information for individual peptides, followed by comparing an in-silico proteolytic digest of an organism's proteome. Typically, only the most abundant peptides are selected for fragmentation (**Figure 4**), whereas data for those peptides in relatively low quantities are not obtained. An inherent problem in shotgun proteomics is identifying proteins of low abundance, such as biomarkers for disease states, against a background of proteins whose concentrations can span up to 12 orders of magnitude.



**Figure 4** Traditional proteomics and Iso-TaG-directed proteomics workflow.

To address the unique challenges of identifying proteins of low abundance, a mass-independent chemical proteomics platform, termed *isotope targeted glycoproteomics* (IsoTaG), was developed by the Carolyn Bertozzi group. The platform is comprised of four central components: (i) metabolic labeling with a chemically functionalized glycan, (ii) chemical tagging and enrichment using an isotopic recoding affinity probe, (iii) directed tandem MS, and (iv) targeted glycopeptide assignment (Figure 4).

IsoTaG is performed by isotopic recoding and enrichment of metabolically labeled glycoproteins followed by directed tandem MS (MS2 or MSn) analysis and intact glycopeptide assignment. Isotopic recoding is accomplished by metabolic labeling of cell or tissue samples with azide- or alkyne-functionalized sugars, followed by chemical conjugation with a biotin probe bearing a unique isotopic signature.

In order to perform isotopic tagging, two IsoTaG probes encoded by zero [M] and two [M + 2] deuterium atoms are required. Probes with different encoding can be used and can be provided by Vector Laboratories though custom synthesis. The IsoTaG probe with zero, and that with two deuterium atoms [M, M + 2], can be used in different proportions; 1:1, 1:2, 1:3 and 1:4. Pattern recognition with isotopic ratio of 1:3 showed the highest fidelity.

Figure 5 Cleavable IsoTaG probe encoded by zero deuterium atoms [M] (R = H) and two deuterium atoms [M+2] (R = D).

Through these probes, a unique isotopic signature is embedded exclusively into the (glyco)peptides. The isotopic signature serves as a computationally recognizable full-scan MS reporter. A computational algorithm, termed *isotopic signature transfer and mass pattern prediction* (IsoStamp), for the detection of recoded species in full-scan mass spectra, was also developed by the Carolyn Bertozzi group. IsoStamp compares observed and predicted isotopic envelopes to identify chemically tagged species in full-scan mass spectra.

IsoTaG has the potential to enhance any proteomics platform that employs chemical labeling for targeted protein identification, including isotope-coded affinity tagging, isobaric tagging for relative and absolute quantitation, and chemical tagging strategies for post-translational modification.

Product #	Description	Pkg. Size
CCT-1448	Click-&-Go® IsoTag Kit for Intact Glycopeptides Profiling *azide modified proteins*	1 kit
CCT-1449	Click-&-Go® IsoTag Kit for Intact Glycopeptides Profiling *alkyne modified proteins*	1 kit
CCT-1450	DADPS H2/D2 Biotin Azide, 2 mg each	1 set
CCT-1451	DADPS H2/D2 Biotin Alkyne, 2 mg each	1 set
CCT-1501	IsoTaG Biotin Azide Pack, 2 mg each	1 set
CCT-1502	IsoTaG Biotin Alkyne Pack, 2 mg each	1 set

#### **Selected References:**

Woo, C.M., et al. (2015). Isotope-targeted glycoproteomics (IsoTaG): a mass-independent platform for intact N- and O-glycopeptide discovery and analysis. Nat Methods., 12: 561 – 7.

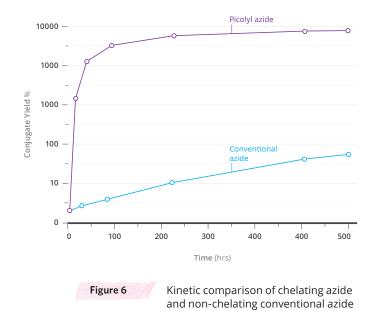
Woo, C. M., et al. (2017). Development of IsoTaG, a Chemical Glycoproteomics Technique for Profiling Intact N- and O-Glycopeptides from Whole Cell Proteomess. J. Proteome Res., 16: 1706 – 18.

Gao, G., et al. (2017). Small Molecule Interactome Mapping by Photoaffinity Labeling Reveals Binding Site Hotspots for the NSAIDs. J. Am. Chem. Soc., **140**: 4259 – 68.

Iso-Tag products are covered by U.S. Patent No.: 10,114,026. This product may be used for research purposes only. It is not licensed for resale and may only be used by the buyer. This product may not be used and is not licensed for clinical assays, where the results of such assays are provided as a diagnostic service. If a diagnostic and therapeutic use is anticipated, then a license must be requested from the University of California. The availability of such diagnostic and therapeutic use license(s) cannot be guaranteed from the University of California.

# **Next Generation Azide Probes**

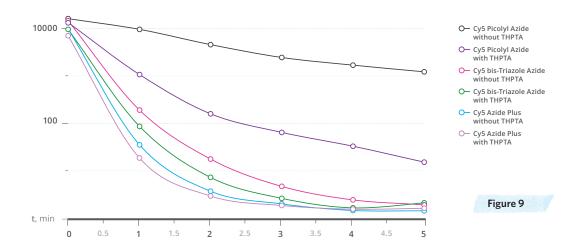
Recent advances in the design of copper-chelating ligands, such as THPTA or BTTAA that stabilize the Cu(I) oxidation state in aqueous solution, improve the kinetics of the coppercatalyzed azide-alkyne cycloaddition (CuAAC) reaction and greatly increase the sensitivity of alkyne detection. Copper-chelating ligands have also been shown to increase the biocompatibility of the CuAAC reaction by preventing the copper ions from causing biological damage<sup>1</sup>. The next step in improving the CuAAC reaction was the development of copper-chelating azides as more reactive substrates. Since it is speculated that the Cu(I)-azide association is the rate-determining step in the CuAAC catalytic cycle<sup>2</sup>, the introduction of a copper-chelating moiety at the azide reporter molecule allows for a dramatic raise of the effective Cu(I) concentration at the reaction site, enhancing the weakest link in the reaction rate acceleration (Figure 7). It has been proposed that the high reactivity of chelating azides comes



from the rapid copper-azido group interaction which occurs prior to Cu(I) acetylide formation, and this renders the deprotonation of alkyne in the rate-determining step<sup>3</sup>. This concept was successfully exploited to perform CuAAC reactions using pyridine-based copper-chelating azides (picolyl azides) as substrates<sup>4-6</sup>. Nevertheless, the copper-chelating motif of picolyl azide molecules is not complete, requiring the presence of a copper chelator (e.g. THPTA) to achieve significant improvement in the kinetics of the CuAAC reaction<sup>3, 4</sup>.

In efforts to improve the performance of the CuAAC reaction in complex media, Vector Laboratories developed new chelating azides with a complete copper-chelating system in their structure, termed "Azides Plus" (Figure 8). These azides are capable of forming strong, active copper complexes and are therefore considered both reactant and catalyst in the CuAAC reaction. Using these types of azides, the CuAAC reaction becomes a bimolecular reaction and displays much faster kinetics compared to the CuAAC reaction performed with conventional azides.

$$\begin{array}{c|c} & & & \\ & & &$$



Comparative kinetic measurements for the CuAAC reaction (Figure 9) were performed using an agarose-alkyne resin labeling experiment (3.0 mM CuSO<sub>4</sub>, with (6.0 mM) or without THPTA ligand) using Cy5 Azide Plus, Cy5 Picolyl Azide, and Cy5 bis-Triazole Azide – the fastest copper-chelating azide that has been reported to date<sup>7</sup>. As expected, the picolyl azide containing the incomplete copper-chelating motif displays relatively slow reactivity, in particular without the presence of THPTA. The kinetic data shows that completing a copper-chelating moiety greatly enhances reactivity, and importantly does not require the presence of copper-chelating ligands. Interestingly, the copper-chelating azides developed by Vector Laboratories display almost identical reactivity in the CuAAC reaction compared to the most reactive copper-chelating azide reported up to now<sup>7</sup>, bis-triazole azide.

The new copper chelating azides allow the formation of azide copper complexes that react almost instantaneously with alkynes under diluted conditions. This unprecedented reactivity in the CuAAC reaction is of special value for the detection of low abundance targets, improving biocompatibility, and any other application where greatly improved S/N ratio is highly desired.

#### **Selected References:**

- 1. Hong, V., et al. (2010). Labeling Live Cells by Copper-Catalyzed Alkyne Azide Click Chemistry. Bioconjugate Chem., 21, 1912-6.
- 2. Rodionov, V.O., et al. (2007). Ligand-accelerated Cu-catalyzed azide-alkyne cycloaddition: A mechanistic report. J. Am. Chem. Soc., 129, 12705–12. Presolski, S.I., et al. (2010). Tailored ligand acceleration of the cu-catalyzed azide-alkyne cycloaddition reaction: Practical and mechanistic implications. J. Am. Chem. Soc., 132, 14570–6.
- 3. Kuang, G.-C., et al. (2011). Experimental investigation on the mechanism of chelation-assisted, copper (ii) acetate-accelerated azide-alkyne cycloaddition. J. Am. Chem. Soc. 133, 13984-4001
- 4. Jiang, H., et al. (2014). Monitoring Dynamic Glycosylation in Vivo Using Supersensitive Click Chemistry. Bioconjugate Chem., 25: 698-706.
- 5. Uttamapinant, C., et al. (2012). Fast, Cell-Compatible Click Chemistry with Copper-Chelating Azides for Biomolecular Labeling. Angew. Chem. Int. Ed., 51: 5852-6.
- 6. Gaebler A., et al. (2016). A highly sensitive protocol for microscopy of alkyne lipids and fluorescently tagged or immunostained proteins. J. Lipid. Res., 57:1934-47.
- 7. Bevilacqua, V., et al. (2014). Copper-Chelating Azides for Efficient Click Conjugation Reactions in Complex Media. Angew. Chem. Int. Ed.,, 53, 5872-6.

Vector Laboratories offers a wide section of fluorescent Azide Plus probes, including AZDyes, Cy Dyes and classic dyes conjugated to azide groups. The photophysical properties of our AZDyes are an exact match to Alexa Fluor® Dyes. The combination of the exceptional reactivity of the azide plus moiety, biocompatibility and brightness of the AZDyes makes these probes of special value not only for the detection of low abundance targets, but also for all other applications where increased S/N ratio is of great value.

Description	Ex/Em	Emission Color	Pkg. Size	Product #
AZDye 350 Azide Plus			1 mg	CCT-1477-1
	346/445	Blue	5 mg	CCT-1477-5
			25 mg	CCT-1477-25
			1 mg	CCT-1474-1
AZDye 405 Azide Plus	402/424	24 Blue	5 mg	CCT-1474-5
			25 mg	CCT-1474-25
	•••••		1 mg	CCT-1475-1
AZDye 488 Azide Plus	494/517	Green	5 mg	CCT-1475-5
			25 mg	CCT-1475-25
			1 mg	CCT-1476-1
AZDye 532 Azide Plus	532/554	Orange	5 mg	CCT-1475-5
		25 mg   CCT-1477     1 mg   CCT-1474     25 mg   CCT-1474     25 mg   CCT-1474     25 mg   CCT-1474     1 mg   CCT-1475     1 mg   CCT-1475     25 mg   CCT-1475     25 mg   CCT-1476     25 mg   CCT-1476     25 mg   CCT-1476     25 mg   CCT-1476     25 mg   CCT-1478     25 mg   CCT-1478     25 mg   CCT-1478     25 mg   CCT-1479     25 mg   CCT-1479     25 mg   CCT-1480     25 mg   CCT-1480     25 mg   CCT-1480     25 mg   CCT-1481     Red   5 mg   CCT-1481     1 mg   CCT-1481     25 mg   CCT-1481     1 mg   CCT-1481     25 mg	CCT-1475-25	
			1 mg	CCT-1478-1
AZDye 546 Azide Plus	554/570	Orange	25 mg CCT-1475-25  1 mg CCT-1478-1  5 mg CCT-1478-5  25 mg CCT-1478-25	
		Orange 5 mg CCT-1478	CCT-1478-25	
	•		1 mg	CCT-1479-1
AZDye 555 Azide Plus	555/572	Red	5 mg	CCT-1479-5
			25 mg	CCT-1479-25
	••••••		1 mg	CCT-1480-1
AZDye 568 Azide Plus	578/602	Red	5 mg	CCT-1480-5
			25 mg	CCT-1480-25
			1 mg	CCT-1481-1
AZDye 594 Azide Plus	590/617	Red	5 mg	CCT-1481-5
			25 mg	CCT-1481-25
			1 mg	CCT-1482-1
AZDye 647 Azide Plus	648/671	Near IR	5 mg	CCT-1482-5
			25 mg	CCT-1482-25

Alexa Fluor  $^{\scriptsize{\circledR}}$  is a registered trademark of Thermo Fisher Scientific.

Description	Ex/Em	Emission Color	Pkg. Size	Product #
			1 mg	CCT-1483-1
PB Azide Plus (Pacific Blue®equivalent)	410/455	Blue	5 mg	CCT-1483-5
			25 mg	CCT-1483-25
			1 mg	CCT-1486-1
TAMRA Azide Plus	553/575	Orange 5 mg CCT-1486	CCT-1486-5	
			25 mg	CCT-1486-25
			1 mg	CCT-1484-1
Cy3 Azide Plus	555/572	Red	Red 5 mg CCT-1484-5	CCT-1484-5
			25 mg	CCT-1484-25
			1 mg	CCT-1485-1
Cy5 Azide Plus	647/663	Near IR	5 mg	CCT-1485-5
			25 mg	CCT-1485-25

Biotin Azide Plus			Catalog#	Unit
CAS:	n/a	O II	CCT-1488-1	1 mg
MW:	582.72	HN NH	CCT-1488-5	5 mg
Solubility:	DMSO, DMF	$\begin{array}{c} \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \end{array} \\ \begin{array}{c} \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\$	CCT-1488-25	25 mg
Description:	Biotinylation reagent with supe kinetics in copper-catalyzed clic		CCT-1488-100	100 mg

Dde Biotin A	Azide Plus		Catalog#	Unit
CAS:	n/a	$N_3 \longrightarrow N \longrightarrow $	CCT-1489-1	1 mg
MW:	815.98	HN NH	CCT-1489-5	5 mg
Solubility:	DMSO, DMF,	THF, DCM	CCT-1489-25	25 mg
Description:	,	eagent with superior per-catalyzed click reactions.		

# Fluorescent Azides

Vector Laboratories offers the largest selection of fluorescent azide probes for click chemistry. Our selection of fluorescent probes includes AZDyes, Cy Dyes and classic dyes conjugated to azide groups. The photophysical properties of our AZDyes are an exact match to Alexa Fluor® Dyes.

Description	Ex/Em	Emission Color	Pkg. Size	Product#
	•••••		1 mg	CCT-1267-1
AZDye 350 Azide	346/445	Blue	5 mg	CCT-1267-5
			25 mg	CCT-1267-25
	•		1 mg	CCT-1307-1
AZDye 405 Azide	402/424	Blue	5 mg	CCT-1307-5
			25 mg	CCT-1307-25
	•		1 mg	CCT-1275-1
AZDye 488 Azide	494/517	Green	5 mg	CCT-1275-5
			25 mg	CCT-1275-25
	•••••		1 mg	CCT-1279-1
AZDye 532 Azide	532/554	Orange	5 mg	CCT-1279-5
			25 mg	CCT-1279-25
			1 mg	CCT-1283-1
AZDye 546 Azide	554/570	Orange 5 mg CCT-1283-	CCT-1283-5	
			25 mg	CCT-1283-25
			1 mg	CCT-1287-1
AZDye 555 Azide	555/572	Red	5 mg	CCT-1287-5
			25 mg	CCT-1287-25
	•		1 mg	CCT-1291-1
AZDye 568 Azide	578/602	Red	5 mg	CCT-1291-5
			25 mg	CCT-1291-25
	••••••		1 mg	CCT-1295-1
AZDye 594 Azide	590/617	Red	5 mg	CCT-1295-5
			25 mg	CCT-1295-25
			1 mg	CCT-1299-1
AZDye 647 Azide	648/671	Near IR	5 mg	CCT-1299-5
			25 mg	CCT-1299-25

Alexa Fluor  $^{\scriptsize \textcircled{\tiny{\$}}}$  is a registered trademark of Thermo Fisher Scientific.

Visit vectorlabs.com for more detailed information

Description	Ex/Em	Emission Color	Pkg. Size	Product #
06.400 A=: I	•••••		1 mg	CCT-1264-1
OG 488 Azide Replacement of Invitrogen's	406/524	C	5 mg	CCT-1264-5
Oregon Green® 488 Azide	496/524	Green	1 mg	
(Catalog number: O10180)			100 mg	CCT-1264-100
			1 mg	CCT-AZ105-1
Caubayyuubadamina 110 Azida	E01/E22	Croop	5 mg	CCT-AZ105-5
Carboxyrhodamine 110 Azide	501/523	Green	25 mg	CCT-AZ105-25
AMRA Azide 553/57			100 mg	CCT-AZ105-100
			1 mg	CCT-AZ109-1
TAMPA Asido	FF2/F7F	Orango	5 mg	CCT-AZ109-5
TAMRA AZIGE	553/5/5	Orange	25 mg	CCT-AZ109-25
			100 mg	CCT-AZ109-100
5 TAMBA A 11			1 mg	CCT-1245-1
5-TAMRA Azide Replacement of Invitrogen's	553/575	Orango	5 mg	CCT-1245-5
Tetramethylrhodamine Azide (Catalog number: T10182).		Orange	25 mg	CCT-1245-25
			100 mg	CCT-1245-100
			1 mg	CCT-AZ119-1
Cv2 A=ido		Red	5 mg	CCT-AZ119-5
Cy3 Azide	553/569	Reu	25 mg	CCT-AZ119-25
			100 mg	CCT-AZ119-100
			1 mg	CCT-AZ118-1
C. T. Ai.d.	C 40 /C74		5 mg	CCT-AZ118-5
Cy5 Azide	649/671	Near IR	25 mg	CCT-AZ118-25
			100 mg	CCT-AZ118-100
			1 mg	CCT-1059-1
C.E.E.A.	670/604		5 mg	CCT-1059-5
Cy5.5 Azide	678/694	Near IR	25 mg	CCT-1059-25
			100 mg	CCT-1059-100
			1 mg	CCT-1052-1
Co.7 A = ido	752/775	N ID	5 mg	CCT-1052-5
Cy7 Azide	753/775	Near IR	25 mg	CCT-1052-25
			100 mg	CCT-1052-100

# Biotin Probes for Click Chemistry

Biotin Azide		Catalog#	Unit
CAS:	n/a oʻ	CCT-1265-5	5 mg
MW:	615.79 HN NH	CCT-1265-25	25 mg
Solubility:	DMSO, DMF, MeOH N <sub>3</sub> OOOOOOOOO	CCT-1265-100	100 mg
Description:	Exact replacement of Ö Ö Northern Sinvitrogen's Biotin Azide (PEG4 carboxamide-6-Azidohexanyl Biotin), Catalog number: B10184		

Biotin-PEG3-Azide		Catalog#	Unit	
CAS:	875770-34-6	Q.	CCT-AZ104-5	5 mg
MW:	444.55	HN	CCT-AZ104-25	25 mg
Solubility:	DMSO, DMF	N <sub>3</sub> OOOON H	CCT-AZ104-100	100 mg
Description:	Biotinylation reagent	0 8	CCT-AZ104-1000	1000 mg

Biotin Azide Plus			Catalog#	Unit
CAS:	n/a	O J	CCT-1488-1	1 mg
MW:	582.72	HN NH H <del>-) (-</del> H H N=N H	CCT-1488-5	5 mg
Solubility:	DMSO, DMF, MeOH	$\langle s \rangle \sim \langle N $	CCT-1488-25	25 mg
Description:	Biotinylation reagent with kinetics in copper-catalyze	•	CCT-1488-100	100 mg

Biotin Picolyl Azide			Catalog#	Unit
CAS:	n/a	∧ N	CCT-1167-5	5 mg
MW:	622.74	N <sub>3</sub> V N O O O O O O O O O O O O O O O O O O	CCT-1167-25	25 mg
Solubility:	DMSO, DMF	H H HN NH	CCT-1167-100	100 mg
Description:	Biotinylation reagent wi	th superior 0		

Dde Biotin P	icolyl Azide	X	Catalog#	Unit
CAS:	n/a	N <sub>3</sub> N O O O O O O O S	CCT-1186-1	1 mg
MW:	815.98	H H H	CCT-1186-5	5 mg
Solubility:	DMSO, DMF, TH	F, DCM	CCT-1186-25	25 mg
Description:	Biotinylation reag	ent with superior		

kinetics in copper-catalyzed click reactions.

Biotin Alkyne			Catalog#	Unit
CAS:	n/a	0	CCT-1266-5	5 mg
MW:	528.26	HN NH	CCT-1266-25	25 mg
Solubility:	DMSO, DMF	H O O O O O O O O O O O O O O O O O O O	CCT-1266-100	100 mg
Description:	Exact replacement of Invitrogen's Biotin Alkyne			

Biotin-PEG4	-Alkyne		Catalog#	Unit
CAS:	1262681-31-1	0	CCT-TA105-5	5 mg
MW:	457.58	»  N  S  N  N	CCT-TA105-25	25 mg
Solubility:	DMSO, DMF, MeOH	H HN NH	CCT-TA105-100	100 mg
Description:	Biotinylation reagent	0	CCT-TA105-1000	1000 mg

# **Biotin Probes for Cu-free Click Chemistry**

(PEG4 carboxamide-Propargyl Biotin),

Catalog number: B10185

DBCO-PEG4	-Biotin		Catalog#	Unit
CAS: MW:	1255942-07-4 749.92		CCT-A105-5 CCT-A105-25	5 mg 25 mg
Solubility: Description:	DMSO, DMF, THF, MeOH Biotinylation reagent	o N N N N N N N N N N N N N N N N N N N	CCT-A105-100	100 mg

WS DBCO-E	Biotin		Catalog#	Unit
CAS:	1363444-70-5		CCT-A116-1	1 mg
MW:	653.77		CCT-A116-5	5 mg
Solubility:	DMSO, DMF, THF, DCM	ON H H	CCT-A116-25	25 mg
Description:	Water-soluble biotinylation reagent	HN NH	CCT-A116-100	100 mg

# Fluorescent Alkyne Probes

Vector Laboratories offers a wide section of fluorescent alkyne probes covering the entire UV-Vis spectrum. Our selection of fluorescent probes includes AZDyes, Cy Dyes and classic dyes conjugated to terminal alkynes. The photophysical properties of our AZDyes are an exact match to Alexa Fluor® Dyes.

Description	Ex/Em	Emission Color	Pkg. Size	Product #
			1 mg	CCT-1269-1
AZDye 350 Alkyne	346/445	Blue	5 mg	CCT-1269-5
			25 mg	CCT-1269-25
			1 mg	CCT-1309-1
AZDye 405 Alkyne	402/424	Blue	5 mg	CCT-1309-5
			25 mg	CCT-1309-25
			1 mg	CCT-1277-1
AZDye 488 Alkyne	494/517	Green	5 mg	CCT-1277-5
			25 mg	CCT-1277-25
			1 mg	CCT-1281-1
AZDye 532 Alkyne	532/554	Orange	5 mg	CCT-1281-5
			25 mg	CCT-1281-25
	554/570		1 mg	CCT-1285-1
AZDye 546 Alkyne		Orange	5 mg	CCT-1285-5
			25 mg	CCT-1285-25
			1 mg	CCT-1289-1
AZDye 555 Alkyne	554/569	Red	5 mg	CCT-1289-5
			25 mg	CCT-1289-25
			1 mg	CCT-1293-1
AZDye 568 Alkyne	578/602	Red	5 mg	CCT-1293-5
			25 mg	CCT-1293-25
			1 mg	CCT-1297-1
AZDye 594 Alkyne	590/617	Red	5 mg	CCT-1297-5
			25 mg	CCT-1297-25
			1 mg	CCT-1301-1
AZDye 647 Alkyne	648/671	Near IR	5 mg	CCT-1301-5
			25 mg	CCT-1301-25

Visit vectorlabs.com for a full list of fluorescent terminal alkynes

Alexa Fluor® is a registered trademark of Thermo Fisher Scientific.

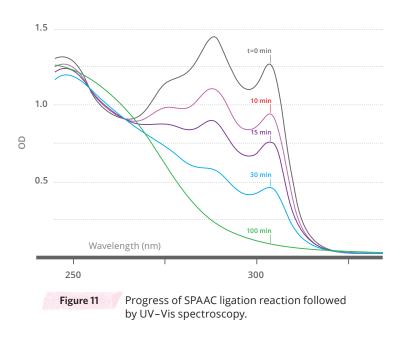
# **Cu-Free Click Chemistry**

The strain-promoted alkyne-azide cycloaddition reaction, also termed the Cu-free click reaction, is a bioorthogonal reaction utilizing a pair of reagents – cyclooctynes and azides – that exclusively and efficiently react with each other while remaining inert to naturally occurring functional groups such as amines (**Figure 10**). SPAAC enables labeling of a wide variety of biomolecules without any auxiliary reagents in an aqueous and otherwise complex chemical environment through the formation of a stable triazole.

Among the large number of known cyclooctynes, the so-called DBCO (dibenzocyclooctynes) compounds comprise a class of reagents that possesses reasonably fast kinetics and good stability in aqueous buffers. Within physiological temperature and pH ranges, the DBCO group will not react with amines or hydroxyls that are naturally present in many biomolecules. Additionally, reaction of the DBCO group with the azide group is significantly faster than with sulfhydryl groups (-SH, thiol).

Unlike many other cyclooctynes, DBCO reagents possess an embedded chromophore that allows for the simple and non-destructive spectroscopic identification of DBCO-containing compounds. This chromophore can also be used for spectroscopic estimation of total incorporated DBCO molecules into a biopolymer.

Another important feature of DBCO compounds is that the progress of SPAAC ligation can be followed in real time by simple UV-Vis spectroscopy. As the "click reaction" progresses the signature an absorbance band at 310 nm disappears as illustrated **Figure 11**.



# Fluorescent Probes for Copper-free Click Chemistry

In applications where the presence of copper is a concern, probes that react with azides via a copper-free click chemistry reaction to form stable triazoles are an ideal alternative to copper-requiring fluorescent alkynes. We offer the largest selection of fluorescent probes for copper-less azide imaging, covering the entire UV-Vis spectrum. Our selection of fluorescent probes includes AZDyes, Cy Dyes and classic dyes conjugated to DBCO alkynes.

Description	Ex/Em	Emission Color	Pkg. Size	Product #
			1 mg	CCT-1310-1
AZDye 405 DBCO	402/424	Blue	5 mg	CCT-1310-5
			25 mg	CCT-1310-25
			1 mg	CCT-1274-1
AZDye 430 DBCO	430/537	Green	5 mg	CCT-1274-5
			25 mg	CCT-1274-25
			1 mg	CCT-1278-1
AZDye 488 DBCO	494/517	Green	5 mg	CCT-1278-5
			25 mg	CCT-1278-25
			1 mg	CCT-1282-1
AZDye 532 DBCO	532/554	Orange	5 mg	CCT-1282-5
			25 mg	CCT-1282-25
	554/570		1 mg	CCT-1286-1
AZDye 546 DBCO		Orange	5 mg	CCT-1286-5
			25 mg	CCT-1286-25
			1 mg	CCT-1290-1
AZDye 555 DBCO	555/572	Red	5 mg	CCT-1290-5
			25 mg	CCT-1290-25
			1 mg	CCT-1294-1
AZDye 568 DBCO	578/602	Red	5 mg	CCT-1294-5
			25 mg	CCT-1294-25
	•		1 mg	CCT-1298-1
AZDye 594 DBCO	590/617	Red	5 mg	CCT-1298-5
			25 mg	CCT-1298-25
			1 mg	CCT-1302-1
AZDye 647 DBCO	648/671	Near IR	5 mg	CCT-1302-5
			25 mg	CCT-1302-25

# Biotin Probes and Labeling Reagents for Copper-free Click Chemistry

DBCO-PEG4	I–Biotin	Catalog#	Unit
CAS: MW: Solubility: Description:	1255942-07-4 749.92 DMSO, DMF, THF, MeOH Biotinylation reagent	CCT-A105-5 CCT-A105-25 CCT-A105-100	5 mg 25 mg 100 mg
WS DBCO-B	liotin	Catalog#	Unit
CAS: MW: Solubility: Description:	1363444-70-5 653.77  DMSO, DMF, THF, DCM  Water-soluble biotinylation reagent	CCT-A116-1 CCT-A116-5 CCT-A116-25 CCT-A116-100	1 mg 5 mg 25 mg 100 mg
DBCO- <i>Sulfo</i>	-NHS Ester	Catalog#	Unit
CAS: MW: Solubility: Description:	1400191-52-7 532.50 Water, DMSO, DMF Water-soluble labeling reagent	CCT-A124-10 CCT-A124-25 CCT-A124-100 CCT-A124-500	10 mg 25 mg 100 mg 500 mg
DBCO-PEG5	5–NHS Ester	Catalog#	Unit
CAS: MW: Solubility: Description:	1378531-80-6 693.74 DMSO, DMF, DCM, THF Labeling reagent with improved stability and solubility	CCT-A102P-2 CCT-A102P-10 CCT-A102P-100 CCT-A102P-500 CCT-A102P-1G	4×2 mg 10 mg 25 mg 100 mg 500 mg 1 g
Sulfo DBCO-	-Maleimide	Catalog#	Unit
CAS: MW: Solubility: Description:	n/a 578.59 Water, DMSO, DMF, DCM, THF Water-soluble, sulfhydryl-reactive labeling reagent	CCT-1230-10 CCT-1230-25 CCT-1230-100 CCT-1230-500	10 mg 25 mg 100 mg 500 mg
Sulfo DBCO-	-PEG4-Maleimide	Catalog#	Unit
CAS: MW: Solubility: Description:	n/a 825.89  Water, DMSO, DMF, DCM, THF  Water-soluble, sulfhydryl-reactive labeling reagent with hydrophilic PEG <sub>4</sub> spacer arm	CCT-1231-10 CCT-1231-25 CCT-1231-100 CCT-1231-500	10 mg 25 mg 100 mg 500 mg

# **Enrichment Media and Kits**

## Click-&-Go® Protein Enrichment Kits (Biotin-Streptavidin Free)

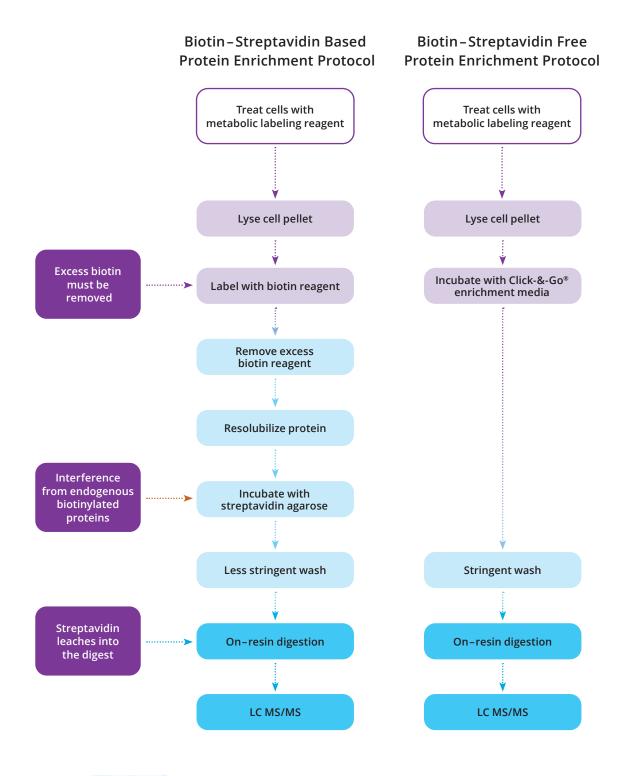
The ability to detect and characterize newly synthesized proteins, changes in spatial or temporal protein expression patterns, or protein degradation resulting from disease, drug treatments, or environmental changes, is an important parameter in cytotoxicity measurements. In most published studies<sup>1</sup>, azide- or alkyne-metabolically labeled, newly synthesized proteins were isolated from a pre-existing poll of proteins by an in-solution click reaction with biotin-alkyne or biotin-azide followed by capture on streptavidin resin. It was reported that with using such a strategy, newly synthesized proteins comprised only 10-20% of the isolated proteins<sup>2</sup>. To address this shortcoming of biotin-streptavidin based enrichment Vector Laboratories has developed an enrichment protocol that allows for direct, covalent capture of alkyne/azide tagged proteins onto azide- or alkyne-modified agarose resin followed by stringent washes to remove nonspecific resin-bound proteins prior to digestion and LCMS analysis.

Another recent study<sup>3</sup> assessed the level of the non-labeled proteins in BONCAT samples by performing the alkyne-based BONCAT sample preparation using HEK-TrKB cells that were labeled with AHA and comparing these results to a control experiment in which the same sample preparation was performed with the same amount of lysate from unlabeled cells. Both the BONCAT and the control samples were analyzed by LCMS. This study consistently identified dramatically more peptides from the BONCAT samples (2371, 2578, and 2681) than from the control samples (69, 19 and 83) at 1% FDR. Moreover, the peptides from the control samples generally had very low signals compared to those from the BONCAT samples. This result shows that the alkyne resin-based enrichment method has minimal contamination from non-AHA-labeled proteins and can be used to isolate high-purity nascent proteomes.

Direct, covalent capture of azide- or alkyne-tagged proteins onto agarose resin represents a substantial improvement compared to the biotin tag-based approach. This is ideal for the covalent capture of specific sub-classes of proteins which have been metabolically, enzymatically, or chemically azido-or alkyne-tagged onto a resin via Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC). The resin containing the covalently attached proteins can be washed with high stringency, virtually eliminating any non-specifically bound proteins without causing loss of target proteins. Upon protease digestion, it yields a highly specific peptide pool that is ideal for mass spectroscopy (e.g., LC MS/MS) based analysis.

## References:

- 1. (a) Shen, W., et al. (2014) Acute synthesis of CPEB is required for plasticity of visual avoidance behavior in Xenopus. Cell Rep., 6: 737 47. (b) Lu, Y. Y., et al. (2014) Prometastatic GPCR CD97 is a direct target of tumor suppressor microRNA-126. ACS Chem. Biol., 9: 334 8. (c) Eichelbaum, K., et al. (2012) Selective enrichment of newly synthesized proteins for quantitative secretome analysis. Nat. Biotechnol., 30: 984 90. (d) Bagert, J. D., et al. (2014) Quantitative, time-resolved proteomic analysis by combining bioorthogonal noncanonical amino acid tagging and pulsed stable isotope labeling by amino acids in cell culture. Mol. Cell Proteomics, 13: 1352 8. (e) Choi, K. Y., et al. (2012) Defining TNF-alpha and IL-1beta induced nascent proteins: combining bioorthogonal non-canonical amino acid tagging and proteomics. J. Immunol. Methods, 382; 189 95. (f) Hodas, J. J., et al (2012) Dopaminergic modulation of the hippocampal neuropil proteome identified by bioorthogonal noncanonical amino acid tagging (BONCAT). Proteomics, 12; 2464 76.
- 2. Howden, A. J., et al. (2013) QuaNCAT: quantitating proteome dynamics in primary cells. Nat. Methods, 10: 343 6.
- 3. Zhang G., et al. (2014) In-Depth Quantitative Proteomic Analysis of de Novo Protein Synthesis Induced by Brain-Derived Neurotrophic Factor. J. Proteome Res., 13, 5707 –14.



**Figure 12** Schematic representation of pull-down workflows for biotin-streptavidin based and biotin-streptavidin free enrichment protocols.



# Click-&-Go® Protein Enrichment Kits, non-cleavable

The Click-&-Go® Protein Enrichment Kit is an efficient tool for covalent capture of azido- or alkyne-tagged proteins on a alkyne- or azide-agarose resin. The kit contains specially formulated components to both catalyze the click reaction and prevent non-specific binding to the alkyne- or azide modified resins. The alkyne- or azide-modified proteins, or their post-translationally modified forms, are captured from complex protein extracts on the azide-alkyne resin supplied. Once covalently attached to the resin via copper-catalyzed click chemistry, the beads can be washed with the highest stringency, virtually eliminating any non-specifically bound proteins to yield a highly enriched population of nascent molecules. Upon protease digestion, this yields a highly pure peptide pool that is ideal for mass spectrometry (e.g., LC MS/MS) based analysis.

Product#	Description	Pkg. Size
CCT-1039	Click-&-Go Protein Enrichment Kit *for capture of alkyne-modified proteins*	1 kit
CCT-1033	Click-&-Go Protein Enrichment Kit *for capture of azide-modified proteins*	1 kit
CCT-1235	Click-&-Go Plus Protein Enrichment Kit *for capture of alkyne-modified proteins*	1 kit

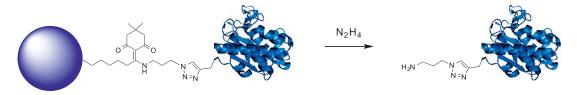
# **Click Functionalized Agarose**

Product #	Description	Pkg. Size
	2 mL	CCT-1032-2
Alkyne agarose resin, 50% slurry	25 mL	CCT-1032-25
	2 mL	CCT-1038-2
Azide agarose resin, 50% slurry	25 mL	CCT-1038-25

Click Functionalized Magnetic Beads are also available, please visit Vector Laboratories website

## Click-&-Go® Dde Protein Enrichment Kits

The Dde linker is stable towards acidic or basic conditions, generally applied buffer systems, and reactive species that are present in a cell extract. It also can withstand harsh wash conditions in order to virtually eliminate any non-specifically bound proteins. The captured proteins can be chemoselectively released under mild aqueous buffered conditions with 2% hydrazine to yield a highly enriched population of intact proteins.



**Figure 14** Schematic representation of hydrazine induced release.

Product #	Description	Pkg. Size
CCT-1153	Click-&-Go Dde Protein Enrichment Kit *for capture and release of alkyne-modified proteins*	1 kit
CCT-1152	Click-&-Go Dde Protein Enrichment Kit *for capture and release of azide-modified proteins*	1 kit



# Click-&-Go® Enrichment Kits for Click Chemistry

The Click-&-Go® Protein Capture Kit provides all of the necessary reagents to perform a conventional capture of azide- or alkyne modified proteins though click labeling with a biotin reagent followed by capture on high-capacity streptavidin agarose resin. The kit includes specially formulated components to perform copper-catalyzed click reactions and subsequent capture on high-capacity streptavidin agarose. Sufficient material is supplied for 25 enrichments based on the provided protocol. The kit provides azide/alkyne labeled BSA as a positive control.

Product#	Description	Pkg. Size
CCT-1441	Click-&-Go Protein Enrichment Kit *for capture of alkyne-modified proteins*	1 kit
CCT-1440	Click-&-Go Protein Enrichment Kit *for capture of azide-modified proteins*	1 kit

## Click-&-Go® DADPS Protein Enrichment Kit for Click Chemistry (acid cleavable)

The Click-&-Go® DADPS Protein Enrichment Kit provides all of the necessary reagents to perform enrichment of azide-modified proteins through conventional biotin-streptavidin affinity purification. The kit includes an acid cleavable DADPS Biotin linker that allows for the release of captured proteins for intact protein analysis or on-beads digestion followed by the release of peptides for subsequent downstream analysis by mass spectrometry. Captured biomolecules can be released under mild conditions, such as 5% aqueous formic acid. Sufficient materials are supplied for 25 enrichments based on the provided protocol below. The kit provides azide labeled BSA as a positive control.

Product#	Description	Pkg. Size
CCT-1443	Click-&-Go DADPS Protein Enrichment Kit *for capture of alkyne-modified proteins*	1 kit
CCT-1442	Click-&-Go DADPS Protein Enrichment Kit *for capture of azide-modified proteins*	1 kit

# Click-&-Go® Dde Protein Enrichment Kit for Click Chemistry (hydrazine cleavable)

The Click-&-Go® Dde Protein Enrichment Kit provides all of the necessary reagents to perform enrichment of alkyne-modified proteins through conventional biotin-streptavidin affinity purification. The kit includes a cleavable Dde Biotin linker that allows for the release of captured proteins for intact protein analysis or on-beads digestion followed by the release of peptides for subsequent downstream analysis by mass spectrometry. Captured biomolecules can be released under mild conditions, such as 2% aqueous hydrazine. Sufficient materials are supplied for 25 enrichments based on the provided protocol. The kit provides alkyne labeled BSA as a positive control.

Product #	Description	Pkg. Size
CCT-1445	Click-&-Go Dde Protein Enrichment Kit *for enrichment of alkyne-modified proteins*	1 kit
CCT-1444	Click-&-Go Protein Enrichment Kit *for enrichment of azide-modified proteins*	1 kit

#### **DADPS Biotin Probes**

The extraordinary strength of the biotin-streptavidin interaction allows for efficient capturing of even highly dilute targets; however, it makes recovery of proteins from affinity resins challenging. Conventional methods to elute biotinylated proteins from immobilized avidin include the following: (i) denaturation of streptavidin by boiling the resin in a denaturing buffer that may include high concentrations of chaotropic salts, (ii) trypsin digestion of proteins while they are bound to the resin, or (iii) elution of proteins with excess free biotin. These protocols can co-elute contaminant proteins by releasing nonspecifically bound proteins and/or naturally biotinylated proteins concurrently with labeled proteins. In addition, some of these methods can cause elution of high levels of resin-based peptides along with the proteins of interest, resulting in further sample contamination.

DADPS (dialkoxydiphenylsilane) Biotin probes eliminate a major limitation of the biotin-streptavidin affinity purification. These reagents contain a biotin moiety linked to an azide moiety through a spacer arm containing a cleavable DADPS linker. Captured biomolecules can be efficiently released under mild conditions (5% or 10% formic acid, 0.5 h) and the small molecular fragment is left on the labeled protein following cleavage. These features make the DADPS probe especially attractive for use in biomolecular labeling and proteomic studies.

DADPS Bioti	n Azide		Catalog#	Unit
CAS:	n/a	Q.	CCT-1330-1	1 mg
MW:	886.19	HN NH	CCT-1330-5	5 mg
Solubility:	DMSO, DMF	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	CCT-1330-25	25 mg
Description:	Cleavable biotinylation reage	0		

DADPS Bioti	in Alkyne		Catalog#	Unit
CAS:	n/a		CCT-1331-1	1 mg
MW:	827.12	HN NH	CCT-1331-5	5 mg
Solubility:	DMSO, DMF	H H H Ph Ph	CCT-1331-25	25 mg
Description:	Cleavable biotinylation reagent			

#### **Selected References:**

- 1. Szychowski, J., et al. (2010). Cleavable Biotin Probes for Labeling of Biomolecules via Azide Alkyne Cycloaddition. J. Am. Chem. Soc., 132: 18351.
- 2. Jinxu, G., et al. (2012). Small Molecule Interactome Mapping by Photoaffinity Labeling Reveals Binding Site Hotspots for the NSAIDs. J. Am. Chem. Soc., 140: 4259.
- 3. Wang, J., et al. (2015). Mapping sites of aspirin-induced acetylations in live cells by quantitative acid-cleavable activity-based protein profiling (QA-ABPP). Sci. Rep., 5: 7896.

## **Photocleavable Biotin Probes**

A major advantage of the photocleavable (PC) linker over all other cleavable linkers is the reagent-free release of the captured biomolecules from streptavidin. This unique property of the photocleavable (PC) linker has promoted its application as a tool for separating, purifying, and identifying desired target biomolecules. PC probes contain a biotin moiety linked to a 'clickable' group through a spacer arm containing a photocleavable moiety. Captured biomolecules can be efficiently photoreleased, typically >90% in 5-25 minutes using an inexpensive, near-UV, low intensity lamp (e.g. 365 nm lamp at 1-5 mW/cm²).

PC Biotin Az	zide	Catalog#	Unit
CAS:	n/a º	CCT-1119-10	10 mg
MW:	825.37 N <sub>3</sub> N <sub>3</sub> O <sub>4</sub> O <sub>4</sub> H <sub>10</sub> N <sub>1</sub> H <sub>10</sub>	CCT-1119-25 CCT-1119-100	25 mg
Solubility:	DMSO, DMF, THF, DCM	CCT-1119-100	100 mg
Description:	Photocleavable biotinylation reagent		

PC Biotin Al	kyne		Catalog#	Unit
CAS:	n/a	0	CCT-1118-10	10 mg
MW:	780.34	HN NH HIII HIII HIII HIII HIII HIII HIII	CCT-1118-25	25 mg
Solubility:	DMSO, DMF, THF, DCM		CCT-1118-100	100 mg
Description:	Photocleavable biotinylation	reagent		

PC DBCO Bio	otin	Catalog#	Unit
CAS:	n/a	CCT-1120-10	10 mg
MW:	1002.14	CCT-1120-25	25 mg
Solubility:	DMSO, DMF, THF, DCM	чн ССТ-1120-100	100 mg
Description:	Photocleavable biotinylation reagent		

## **Selected References:**

- 1. Wang, Z., et al. (2010). Enrichment and Site Mapping of O-Linked N-Acetylglucosamine by a Combination of Chemical/Enzymatic Tagging, Photochemical Cleavage, and Electron Transfer Dissociation Mass Spectrometry. Mol. Cell. Proteom., 9: 153.
- 2. Pandor, M., et al. (2002). Photochemical Control of the Infectivity of Adenoviral Vectors Using a Novel Photocleavable Biotinylation Reagent. Chemistry & Biology, 9: 567.
- 4. Zhou, G., et al. (2010). Photocleavable Peptide-Conjugated Magnetic Beads for Protein Kinase Assays by MALDI-TOF MS. Bioconjugate Chem., 21: 1917.
- 6. Kim, H., et al. (2009). An Azido-Biotin Reagent for Use in the Isolation of Protein Adducts of Lipid-derived Electrophiles by Streptavidin Catch and Photorelease. Mol. Cell. Proteom., 8: 2080.

8. Szychowski, J., et al. (2010). Cleavable Biotin Probes for Labeling of Biomolecules via Azide-Alkyne Cycloaddition. J. Am. Chem. Soc., 132: 18351.

#### **Dde Biotin Probes**

$$N_2H_4$$
 $N_2H_4$ 
 $N_2H_4$ 
 $N_1H_2$ 
 $N_2H_4$ 

These novel click chemistry probes for enrichment of azide- or alkyne-tagged biomolecules overcome a major drawback of the biotin-streptavidin affinity purification associated with the extraordinary strength of the biotin-streptavidin interaction. These probes contain a biotin moiety linked to a "clickable" group through a spacer arm containing a Dde linker. The Dde moiety is stable to rigorous denaturing wash conditions, such as basic conditions including generally applied buffer systems to which the biological sample may be exposed. At the same time the Dde linker can be quantitatively cleaved under mild aqueous buffered conditions with 2% hydrazine. Finally, the cleaved moiety that remains on the modified peptide minimally changes the peptide mass and generates an additional positive charge, which facilitates peptide sequencing by ETD.

Dde Biotin A	azide	Catalog#	Unit
CAS:	n/a ×	CCT-1136-1	1 mg
MW:	695.37	CCT-1136-5	5 mg
Solubility:	DMSO, DMF, THF, DCM	CCT-1136-25	25 mg
Description:	Cleavable biotinylation reagent		
Dde Biotin A	zide Plus	Catalog#	Unit
CAS:	n/a ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° ° °	CCT-1489-1	1 mg
MW:	815.98 N <sub>3</sub> N <sub>1</sub> N <sub>2</sub> N <sub>3</sub> N <sub>4</sub> N <sub>2</sub> N <sub>3</sub> N <sub>4</sub>	CCT-1489-5	5 mg
Solubility:	DMSO, DMF, THF, DCM	CCT-1489-25	25 mg
Description:	Next generation copper-chelating biotin probe for CuAAC		
Dde Biotin P	icolyl Azide	Catalog#	Unit
CAS:	n/a N <sub>3</sub> \ N <sub>3</sub> \ O O O O O O O O O O O O O O O O O O	CCT-1186-1	1 mg
MW:	815.98	CCT-1186-5	5 mg
Solubility:	DMSO, DMF, THF, DCM	CCT-1186-25	25 mg
Description:	Biotinylation reagent with superior kinetics in copper-catalyzed click reactions.		
Dde Biotin A	lkyne	Catalog#	Unit

CCT-1137-1

CCT-1137-5

CCT-1137-25

1 mg

5 mg

25 mg

#### **Selected References:**

n/a

650.83

DMSO, DMF, THF, DCM

Cleavable biotinylation reagent

- 1. Yang Y., et al. (2013). Cleavable Trifunctional Biotin Reagents for Protein Labeling, Capture, and Release. Chem. Commun., 48: 5366
- 2. Matthew E.G., et al. (2016) Comprehensive Mapping of O-GlcNAc Modification Sites Using a Chemically Cleavable Tag. Mol. Biosyst. 12: 1756.
- $3. \ \ Gertsik\ N., et\ \emph{al.}\ (2017).\ Mapping\ the\ Binding\ Site\ of\ BMS-708163\ on\ y-Secretase\ with\ Cleavable\ Photoprobes.\ Cell\ Chemical\ Biology,\ \textbf{32}:\ 3.$

CAS:

MW:

Solubility:

Description:

## **Diazo Biotin Probes**

Diazobenzene-based biotin probes can be chemoselectively cleaved in mild aqueous buffered conditions with 100 mM sodium dithionite. The diazobenzene linker is stable towards acidic or basic conditions, including generally applied buffer systems to which the biological sample may be exposed.

Diazo Biotin	Azide		Catalog#	Unit
CAS:	1339202-33-3	0 HO	CCT-1041-1	1 mg
MW:	711.83	HN NH	CCT-1041-5	5 mg
Solubility:	DMSO, DMF		CCT-1041-25	25 mg
Description:	Cleavable biotinylation reagent	Ö		

Diazo Biotin	Alkyne		Catalog#	Unit
CAS:	n/a	O HO	CCT-1042-1	1 mg
MW:	795.54	HN NH	CCT-1042-5	5 mg
Solubility:	DMSO, DMF		CCT-1042-25	25 mg
Description:	Cleavable biotinylation	on reagent		

Diazo DBCO	Biotin		Catalog#	Unit
CAS:	n/a		CCT-1043-1	1 mg
MW:	973.15	О	CCT-1043-5	5 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1043-25	25 mg
Description:	Cleavable biotinylation reagent	0 0		

## **Selected References:**

- 1. Yang Y., et al. (2013). Cleavable Trifunctional Biotin Reagents for Protein Labeling, Capture, and Release. Chem. Commun., 48: 5366
- 2. Yang Y-Y., et al. (2010) Bioorthogonal Chemical Reporters for Monitoring Protein Acetylation. J. Am. Chem. Soc. 132: 3640.
- 3. Rangan K. J., et al. (2010). Rapid visualization and large-scale profiling of bacterial lipoproteins with chemical reporters. J. Am. Chem. Soc, 132: 10628.

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# Metabolic Labeling Reagents

L-Holliopi o	pargylglycine (HPG)		Catalog#	Unit
CAS:	98891-36-2		CCT-1067-25	25 mg
MW:	163.60		CCT-1067-100	100 mg
Solubility:	Water, DMSO, DMF	H <sub>2</sub> N OH	CCT-1067-1000	1000 mg
4-Azido-L-p	henylalanine		Catalog#	Unit
CAS:	33173-53-4	H <sub>2</sub> NCO <sub>2</sub> H	CCT-1406-1G	1 g
MW:	206.20	1.2.1	CCT-1406-5G	5 g
Solubility:	Water, DMSO, DMF	N <sub>3</sub>	CCT-1406-25G	25 g
5-Ethynyl-2	'-deoxyuridine (EdU)		Catalog#	Unit
CAS:	61135-33-9		CCT-1149-25	25 mg
MW:	252.23	NH	CCT-1149-100	100 mg
Solubility:	Water, DMSO, DMF	ĽN,‱O	CCT-1149-500	500 mg
,-		HOOH	CCT-1149-1000	1000 mg
5-Ethynyl U	ridine (5-EU)		Catalog#	Unit
CAS:	69075-42-9	0	CCT-1261-10	10 mg
MW:	268.22	NH	CCT-1261-25	25 mg
Solubility:	Water, DMSO, DMF	но — О	CCT-1261-100	100 mg
		ОНОН	CCT-1261-500	500 mg
F-ara-EdU			Catalog#	Unit
CAS:	n/a	O	CCT-1403-5	5 mg
MW:	270.22	NH L	CCT-1403-25	25 mg
Solubility:	Water, DMSO, DMF	\N^O	CCT-1403-100	100 mg
		HOOL	CCT-1403-500	500 mg
O-proparg	yl-puromycin (OPP)		Catalog#	Unit
CAS:	n/a	Ň	CCT-1407-5	5 mg
MW:	495.54	N N	CCT-1407-25	25 mg
Solubility:	Water, DMSO, DMF	HONNN	CCT-1407-100	100 mg
		O NH OH		
		NH <sub>2</sub>		

L-Azidohom	noalanine (AHA)		Catalog#	Unit
CAS:	942518-29-8	N₃ J	CCT-1066-25	25 mg
MW:	180.59	∫ OH	CCT-1066-100	100 mg
Solubility:	Water, DMF, DMSO	H <sub>2</sub> N $\nearrow$ O	CCT-1066-1000	1000 mg
		Ü	CCT-1066-5G	5 g
N-azidoace	tylmannosamine tetraacylated (Ac	4ManNAz)	Catalog#	Unit
CAS:	361154-30-5	0	CCT-1084-5	5 mg
MW:	430.37	AcO HN N <sub>3</sub>	CCT-1084-25	25 mg
Solubility:	DMSO, DMF, MeOH	AcO 100 OAc	CCT-1084-100	100 mg
N-(4-penty)	noyl)-mannosamine tetraacylated (	Ac4ManNAl)	Catalog#	Unit
CAS:	935658-93-8	-	CCT-1154-5	5 mg
MW:	427.40	0 	CCT-1154-25	25 mg
Solubility:	DMSO, DMF, MeOH	AcO HN OAc	CCT-1154-100	100 mg
N-azidoace	tylglucosamine tetraacylated (Ac4G	GlcNAz)	Catalog#	Unit
CAS:	98924-81-3	OAc	CCT-1085-5	5 mg
MW:	430.37	AcO	CCT-1085-25	25 mg
Solubility:	DMSO, DMF, MeOH	N <sub>3</sub> NH OAc	CCT-1085-100	100 mg
N-(4-pentyı	noyl)-glucosamine tetraacylated (A	c4GlcNAl)	Catalog#	Unit
CAS:	1361993-37-4	_OAc	CCT-1155-5	5 mg
MW:	427.40	Aco	CCT-1155-25	25 mg
Solubility:	DMSO, DMF, MeOH	ACO NH OAC	CCT-1155-100	100 mg
N-azidoace	tylgalactosamine tetraacylated (Ac	4GalNAz)	Catalog#	Unit
CAS:	653600-56-7	OAc OAc	CCT-1086-5	5 mg
MW:	430.37	AcO OAc	CCT-1086-25	25 mg
Solubility:	DMSO, DMF, DCM, THF	NH NH	CCT-1086-100	100 mg
N-(4-pentyı	noyl)-galactosamine tetraacylated (	(Ac4GalNAl)	Catalog#	Unit
CAS:	1658458-26-4	QAc_OAc	CCT-1156-5	5 mg
BANA/.	427.40		CCT-1156-25	25 mg
MW:	727.70	AcO - OAc	CC	238

# Metabolic Labeling Reagents

Kdo Azide			Catalog#	Unit
CAS:	1380099-68-2	N <sub>3</sub> OH) ⊕	CCT-1241-10	10 mg
MW:	280.24	OH HO NH₄	CCT-1241-25	25 mg
Solubility:	DMSO, DMF, Water	HO CO <sub>2</sub> OH	CCT-1241-100	100 m
6-azido-6-d	leoxy-N-acetyl-glucosamin	e triacylated (Ac3-6AzGlcNAc)	Catalog#	Unit
CAS:	n/a	N	CCT-1258-5	5 mg
MW:	372.33	N <sub>3</sub> 0	CCT-1258-25	25 mg
Solubility:	DMSO, DMF, DCM, THF	ACO NHAC OAC	CCT-1258-100	100 m
6-Azide-Tre	halose (6-TreAz)		Catalog#	Unit
CAS:	n/a	OH	CCT-1472-5	5 mg
MW:	367.31	HOLO	CCT-1472-25	25 mg
6 1 1 111	DMCO DME Motor	HO	CCT-1472-100	100 m
Solubility:	DMSO, DMF, Water	HO OH OH	CC1 11/2 100	
		HO OH OH		
O-Alkyne-T	rehalose (O-AlkTMM)	HO OH OH	Catalog#	Unit
O-Alkyne-T	rehalose (O-AlkTMM)	HO OH OH	Catalog# CCT-1473-5	Unit 5 mg
O-Alkyne-T	rehalose (O-AlkTMM)	HO OH OH OH	Catalog#	Unit 5 mg 25 mg
O-Alkyne-T CAS: MW: Solubility:	n/a 464.46 DMSO, DMF, Water	HOO OH OH	Catalog# CCT-1473-5 CCT-1473-25	Unit 5 mg 25 mg
O-Alkyne-T CAS: MW: Solubility:	n/a 464.46 DMSO, DMF, Water	HO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#  CCT-1166-5	Unit 5 mg 25 mg 100 m
O-Alkyne-T CAS: MW: Solubility: Alkynyl Ste	rehalose (O-AlkTMM)  n/a 464.46  DMSO, DMF, Water  aric Acid	HOO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#	Unit 5 mg 25 mg 100 m
O-Alkyne-T CAS: MW:	rehalose (O-AlkTMM)  n/a 464.46  DMSO, DMF, Water  aric Acid  34450-18-5	HO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#  CCT-1166-5	Unit 5 mg 25 mg 100 m
O-Alkyne-T CAS: MW: Solubility: Alkynyl Ste CAS: MW:	rehalose (O-AlkTMM)  n/a 464.46 DMSO, DMF, Water  aric Acid  34450-18-5 280.45 DMSO, DMF, DCM, THF	HOO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#  CCT-1166-5  CCT-1166-25	Unit 5 mg 25 mg 100 m  Unit 5 mg 25 mg
O-Alkyne-To CAS: MW: Solubility: Alkynyl Ste CAS: MW: Solubility:	rehalose (O-AlkTMM)  n/a 464.46 DMSO, DMF, Water  aric Acid  34450-18-5 280.45 DMSO, DMF, DCM, THF	HONO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#  CCT-1166-5  CCT-1166-100  Catalog#  Catalog#	Unit 5 mg 25 mg 100 m  Unit 5 mg 25 mg 100 n
O-Alkyne-Tocas: MW: Solubility:  Alkynyl Stea CAS: MW: Solubility:	rehalose (O-AlkTMM)  n/a 464.46 DMSO, DMF, Water  aric Acid  34450-18-5 280.45 DMSO, DMF, DCM, THF	HOO OH OH OH	Catalog#  CCT-1473-5  CCT-1473-25  CCT-1473-100  Catalog#  CCT-1166-5  CCT-1166-100  Catalog#  Catalog#	Unit 5 mg 100 m  Unit 5 mg 25 mg 100 n

Alkynyl My	ristic Acid		Catalog#	Unit
CAS:	82909-47-5	OH	CCT-1164-5	5 mg
MW:	244.32		CCT-1164-25	25 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1164-100	100 mg
Azido Myris	stic Acid		Catalog#	Unit
CAS:	80667-36-3	N <sub>3</sub> OH	CCT-1345-5	5 mg
MW:	241.33		CCT-1345-25	25 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1345-100	100 mg
Azido Palm	itic Acid		Catalog#	Unit
CAS:	118162-46-2	N <sub>3</sub> OH	CCT-1346-5	5 mg
MW:	283.41		CCT-1346-25	25 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1346-100	100 mg
Alkyne Cho	lesterol		Catalog#	Unit
CAS:	1631985-09-5	HO H H	CCT-1409-1	1 mg
MW:	396.61		CCT-1409-5	5 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1409-25	25 mg
27-Alkyne (	Cholesterol		Catalog#	Unit
CAS:	1527467-07-7	HO H H	CCT-1410-1	1 mg
MW:	396.66		CCT-1410-5	5 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1410-25	25 mg
E-Cholester	rol Alkyne		Catalog#	Unit
CAS:	n/a	H H	CCT-1411-1	1 mg
MW:	396.66		CCT-1411-5	5 mg
Solubility:	DMSO, DMF, DCM, THF		CCT-1411-25	25 mg

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