

Click Chemistry Toolbox **2020/21**

- **EdU Cell Proliferation Assay Kits**
- **Nascent Protein Synthesis Assay Kits**
- **Fluorescent Probes for Cu-free Click Chemistry**
- **Fluorogenic Azides Probes** NEW.
- **Azide Plus Next Generatin Azide Probes**
- **Iso-TaG Reagents and Kits NEW?**
- **Cleavable Click Chemistry Biotin Probes**
- **Click Chemistry Enrichment Kits and Media**

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 azidealkyne 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.

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).

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).

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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.

Introduction

Measuring cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anti-cancer drugs. The most accurate methods rely on directly measuring DNA synthesis. Traditionally, this was performed by incorporating nucleoside analogs like [³H]thymidine or 5-bromo-2'-deoxyuridine (BrdU) to cells during replication, and their incorporation into DNA is detected or visualized by autoradiography or an antibody-based detection of the nucleoside analog bromo-deoxyuridine (BrdU). Both methods exhibit several limitations. Working with [3 H]thymidine is troublesome because of its radioactivity. Autoradiography is slow and is thus not suitable for rapid high-throughput studies. The major disadvantage of BrdU staining is that the double-stranded DNA blocks the access of the anti-BrdU antibody to BrdU units. Therefore, samples must be subjected to harsh denaturing conditions resulting in degradation of the structure of the specimen.

Click-&-Go™ EdU Cell Proliferation assays are novel alternatives to the [3H]thymidine and BrdU assays for directly measuring DNA synthesis. EdU (5-ethynyl-2´-deoxyuridine) is a nucleoside analog to thymidine, containing the ethynyl moiety that is efficiently incorporated into DNA during active DNA synthesis. The detection of the alkyne found in the ethynyl moiety of EdU is based on a fast, highly specific click reaction using best-in-class (Alexa Fluor® equivalents) fluorescent azide dyes. In contrast to BrdU assays, Click-&-Go™ EdU Cell Proliferation assays are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. Furthermore, the streamlined detection protocol reduces the total number of steps and significantly decreases the total amount of required time. The simple click chemistry detection procedure is complete within 30 minutes and is compatible with multiplexing for content and context-rich results.

Click-&-Go™ EdU Cell Proliferation Kit for Imaging

The Click-&-Go™ EdU Cell Proliferation Kit is a superior alternative to traditional proliferation assays and is optimized for fluorescence microscopy applications.

The kit includes blue fluorescent Hoechst 33342 dye for performing cell cycle analysis on samples from adherent cells. A sufficient amount of reagents is provided for imaging 50 (18×18) coverslips using 500 μ L of reaction buffer per test.

Click-&-Go™ EdU Flow Cytometry Kits

The Click-&-Go™ EdU Flow Cytometry Kit is optimized for DNA replication analysis in proliferating cells using standard flow cytometry methods. The kit contains all of the components needed to detect the incorporated alkynes, including aldehyde-based fixation and detergent permeabilization reagents. A sufficient amount of reagents is provided for 50 or 100 assays based on the protocol provided.

Click-&-Go™ Plus EdU Proliferation Assays

Click-&-Go™ Plus EdU is one of the next step in improving the biocompatibility and sensitivity of traditional Click-&- Go™ proliferation assays. The copper concentrations typically used in traditional click chemistry reactions can affect fluorophores such as green fluorescent protein, mCherry, and R-phycoerythrin and can result in loss of fluorescence signal. Click-&-Go Plus EdU assays employ the newest generation of copper-chelating azides, Azide Plus probes, that form a strong copper-azide complex and thus dramatically raise the copper concentration at the reaction site without the need to maintain overall copper concentration at high level (**Figure 3**). This copper complex reacts almost instantaneously with alkynes under diluted conditions. The use of innovative copper-chelating azides allows for employing low copper concentration in Click-&-Go™ Plus EdU assays during copper-catalyzed detection reaction, enabling DNA synthesis–based cell proliferation assays that are compatible with GFP multiplex imaging or flow cytometry experiments.

Click-&-Go™ Plus EdU Cell Proliferation Kit for Imaging

Click-&-Go™ Plus EdU Cell Proliferation Kits utilize the newest generation of copper-chelating azides that allow for the detection of newly synthesized DNA though a copper-catalyzed click reaction at low copper concentrations. Because of the mild reaction conditions, the Click-&-Go Plus EdU assays can accurately determine cell proliferation while preserving cell morphology, DNA integrity, antigen binding sites, and the fluorescent signal from GFP. Preservation of DNA integrity allows for DNA staining, including staining with dyes used for cell cycle analysis.

Click-&-Go™ Plus EdU Cell Proliferation Kit for Flow Cytometry

The Click-&-Go™ Plus EdU Flow Cytometry Kit is optimized for DNA replication analysis in proliferating cells using standard flow cytometry methods. The kit utilizes the newest generation of fluorescent azides for detection of EdU incorporated into newly synthesized DNA. These fluorescent azides contain a complete copper-chelating system in their structure (**Figure 3**). 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 greatly improves the sensitivity of cell proliferation assays. A sufficient amount of reagents is provided for 50 or 100 assays based on the protocol provided.

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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., methioninecontaining) 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.

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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 Cu-catalyzed 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.

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.

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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 Click Chemistry Tools 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.

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.

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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 copper-catalyzed 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 damage¹. The next step in improving the CuAAC reaction was the development of copperchelating azides as more reactive substrates. Since it is speculated that the Cu(I)-azide association is the rate-determining step in the CuAAC catalytic cycle², 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 copperazido group interaction which occurs prior to Cu(I) acetylide formation, and this renders the deprotonation of alkyne in the rate-determining step 3 . This concept was successfully exploited to perform CuAAC reactions using pyridinebased copper-chelating azides (picolyl azides) as substrates⁴⁻⁶. 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^{3, 4}.

In efforts to improve the performance of the CuAAC reaction in complex media, Click Chemistry Tools 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.

Comparative kinetic measurements for the CuAAC reaction **(Figure 9)** were performed using an agarose-alkyne resin labeling experiment (3.0 mM CuSO₄, 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 date⁷. 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 Click Chemistry Tools display almost identical reactivity in the CuAAC reaction compared to the most reactive copper-chelating azide reported up to now⁷, 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:

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- 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.
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Click Chemistry Tools offers a wide section of fluorescent Azide Plus probes, including AFDyes, Cy Dyes and classic dyes conjugated to azide groups. The photophysical properties of our AFDyes are an exact match to Alexa Fluor® Dyes. The combination of the exceptional reactivity of the azide plus moiety, biocompatibility and brightness of the AFDyes 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.

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kinetics in copper-catalyzed click reactions.

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

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Visit www.clickchemistrytools.com to browse our selection of fluorescent azide probes

Biotin Picolyl Azide Catalog# Unit Price

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Biotin Probes for Cu-free Click Chemistry

reagent

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

Visit www.clickchemistrytools.com for a full list of fluorescent terminal alkynes

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**.

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 AFDyes, Cy Dyes and classic dyes conjugated to DBCO alkynes.

Visit www.clickchemistrytools.com to browse a full list of fluorescent probes for Cu-free click chemistry.

Biotin Probes and Labeling Reagents for Copper-free Click Chemistry

WS DBCO – Biotin Catalog# Unit Price

Full list of DBCO reagents available at www.clickchemistrytools.com

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 studies¹, azide– or alkyne–metabolically labeled, newly synthesized proteins were isolated from a pre-existing poll of proteins by an in-solution click reaction with biotinalkyne 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 proteins². To address this shortcoming of biotinstreptavidin based enrichment Click Chemistry Tools 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 study³ assessed the level of the non-labeled proteins in BONCAT samples by performing the alkynebased 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 posttranslationally 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.

Click Functionalized Agarose

Click Functionalized Magnetic Beads are also available, please visit CCT website

Click-&-Go™ PC Protein Enrichment Kits

Photolabile linkers are stable towards various conditions (acidic/basic, including generally applied buffer systems) to which the biological sample may be exposed. The cleavage of photoliable probes is achieved with near-UV (365 nm) irradiation using an inexpensive, low intensity lamp that can be found virtually in every laboratory. Selective, reagentfree release conditions and high stability in various chemical conditions are great advantages of photolabile capture and release kits.

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 nonspecifically 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.

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.

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 azidemodified 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.

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 alkynemodified 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.

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.

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biotinylation reagent

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365 nm

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).

Selected References:

Photocleavable Biotin Probes

- 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.
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- 3. Zhou, G., *et al.* (2010). Photocleavable Peptide-Conjugated Magnetic Beads for Protein Kinase Assays by MALDI-TOF MS. *Bioconjugate Chem.,* **21**: 1917.
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- 5. 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

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 biotinstreptavidin 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.

Solubility: DMSO, DMF, THF, DCM $\left(\sqrt{N}\right)^{1/2}$ $\left(\sqrt{N}\right)^{1/2}$ $\left(\sqrt{N}\right)^{1/2}$ $\left(\sqrt{N}\right)^{1/2}$ 1137–100 100 mg \$1095

Selected References:

Description: 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 al.* (2017). Mapping the Binding Site of BMS-708163 on y-Secretase with Cleavable Photoprobes. *Cell Chemical Biology*, **32**: 3.

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.

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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