

Click-iT™ Glycoprotein Labeling and Detection Products— Enabling Tools for Advancing the Glycoproteome

The Click-iT™ glycoprotein labeling and detection products provide a simple and robust two-step technique (Figure 1) to detect and characterize *O*-linked, sialic acid-containing or *O*-GlcNAc-modified glycoproteins in the low femtomole range (Figure 2). In step one, azide-modified sugars are incorporated into protein glycan structures with either a Click-iT™ metabolic labeling reagent or Click-iT™ Enzymatic Labeling Kit (Figure 1, Table 1). In step 2, utilizing the chemoselective ligation or “click” reaction between an azide and an alkyne, the azido-labeled proteins are detected with a Click-iT™ Glycoprotein Detection kit for gels or western blots (Table 2). Click-iT™ products are compatible with downstream LC-MS/MS and MALDI MS analyses and can be used for differential analyses of glycoprotein subclasses, total glycoproteins, phosphoproteins, and total proteins (Table 3).

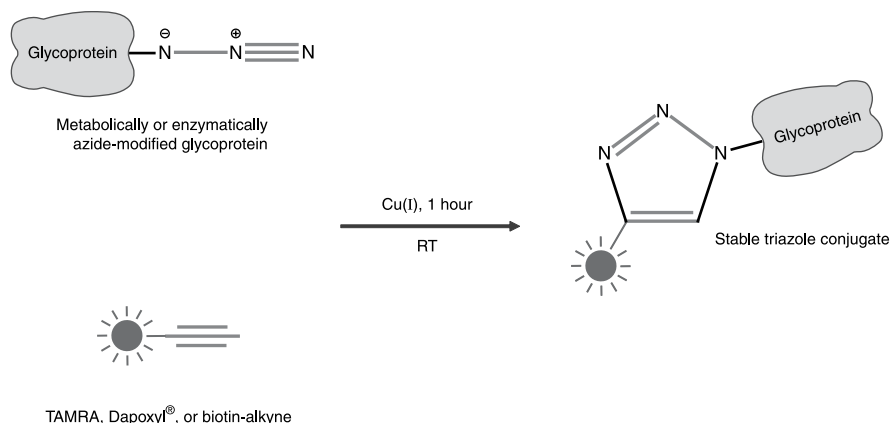


Figure 1. Click-iT™ azide/alkyne reaction.

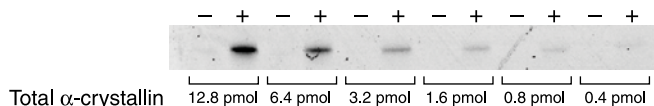


Figure 2. Dilution series of α -crystallin labeled with the Click-iT™ *O*-GlcNAc Enzymatic Labeling System. Detection was performed using the Click-iT™ TAMRA Alkyne Detection Reagent (+). Alternate lanes contain α -crystallin subjected to the same procedure without the addition of the Gal-T1 (Y289L) enzyme (-). Only 2% to 10% of the total α -crystallin is *O*-GlcNAc modified, translating to 8–10 fmol and demonstrating the sensitivity of this detection technology.

Table 1. Click-iT™ Glycoprotein Labeling Products.

Sample	Glycoprotein subclass	Click-iT™ Labeling Product	Catalog Number
Cultured Cells	O-linked	Click-iT™ GalNAz Metabolic Glycoprotein Labeling Reagent	C33365
	O-GlcNAc	Click-iT™ GlcNAz Metabolic Glycoprotein Labeling Reagent	C33367
	Sialic acid	Click-iT™ ManNAz Metabolic Glycoprotein Labeling Reagent	C33366
Pure protein, cell lysate or protein extract	O-GlcNAc	Click-iT™ O-GlcNAc Enzymatic Labeling System	C33368

Table 2. Analytical method compatibility of Click-iT™ Glycoprotein Detection Kits.

Detection Method	Click-iT™ Glycoprotein Detection Kit	Catalog Number
1D or 2D gel	Click-iT™ Tetramethylrhodamine (TAMRA) Glycoprotein Detection Kit	C33370
	Click-iT™ Dapoxyl® Glycoprotein Detection Kit	C33371
Western Blot	Click-iT™ Biotin Glycoprotein Detection Kit	C33372
Mass Spectrometry	Click-iT™ Tetramethylrhodamine (TAMRA) Glycoprotein Detection Kit	C33370
	Click-iT™ Dapoxyl® Glycoprotein Detection Kit	C33371
	Click-iT™ Biotin Glycoprotein Detection Kit	C33372

Table 3. Multiplexed Proteomics™ Compatibility.

Click-iT™ Detection Reagent	SYPRO® Ruby protein gel stain	Pro-Q® Emerald glycoprotein gel stain	Pro-Q® Diamond phosphoprotein gel stain
Click-iT™ TAMRA (C33370)	X	X	
Click-iT™ Dapoxyl® (C33370)	X		X

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