Click-iT™ Protein Analysis Detection Kits

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
TAMRA alkyne* (Component A) (in C33370) or Dapoxyl® alkyne* (Component A) (in C33371) or Biotin alkyne (Component A) (in C33372)	70 µL in DMSO	ND	• ≤–20˚C • Desiccate • Protect from light	When stored as directed the kit is stable for at least 6 months.
Click-iT™ reaction buffer, (Component B)	1.2 mL	2X	• ≤6˚C	
$CuSO_4$ (Component C)	200 µL	40 mM		
Click-iT™ reaction buffer additive 1 (Component D)	10 vials	NA	• ≤–20˚C • Desiccate	
Click-iT™ reaction buffer additive 2 (Component E)	1 vial			
Number of reactions: Sufficient material is supplied for 10 reactions, based on the protocol below.				
* TAMRA and Dapoxyl® reagents may be handled in normal room light, but avoid prolonged exposure to light. ND = Not disclosed. NA = Not applicable.				

Introduction

The Click-iT™ Protein Analysis Detection Kits utilize the chemoselective ligation or "click" reaction between an azide and an alkyne[1] to sensitively detect new protein synthesis or subclasses of glycoproteins modified with an azide group (Figure 1). Azide-modified sugars can be incorporated into protein glycan structures metabolically (C33365, C33366, C33367) or enzymatically (C33368); for nascent protein synthesis the azide-modified amino acid (L-AHA, Invitrogen Cat. no C10102) can be metabolically incorporated into proteins (Table 2).

Detection sensitivity in 1D gels (Figure 2) and western blots is in the low femtomole range. The detection method is compatible with downstream mass spectrometry (MS) analyses including LC-MS/MS and MALDI MS and can be used for differential analyses of glycoprotein subclasses, total glycoproteins, phosphoproteins, nascent proteins, and total proteins. Following electrophoresis, TAMRA-labeled samples can be post-stained with Pro-Q® Emerald 300 Glycoprotein gel stain and/or SYPRO® Ruby protein gel stain, while Dapoxyl®-labeled samples can be post-stained with Pro-Q® Diamond phosphoprotein gel stain and/or SYPRO® Ruby protein gel stain (Figure 2). TAMRA and biotin-labeled samples can be detected before or after probing the western blot with a primary antibody (Table 3).

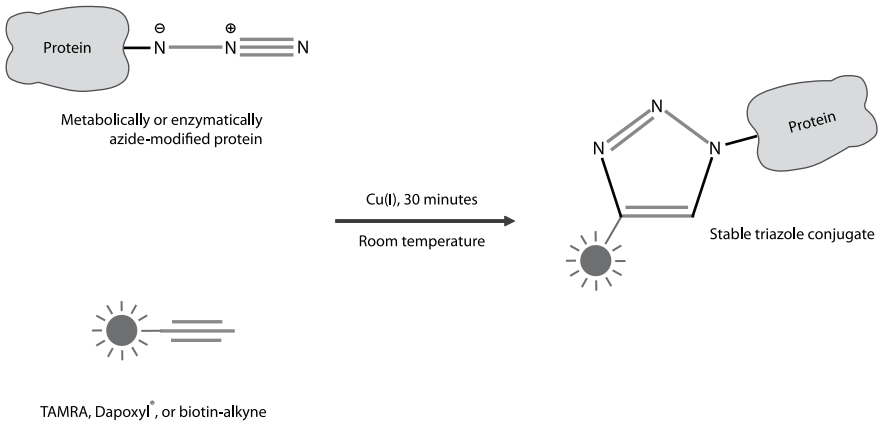

Figure 1. Click-iT™ azide/alkyne reaction.

Table 2. Click-iT™ Protein Labeling Reagents.

Sample	Labeled product	Click-iT™ labeling reagent
Cultured Cells	*O*-linked glycoproteins	Click-iT™ GalNAz Metabolic Glycoprotein Labeling Reagent (C33365)
	New proteins	Click-iT™ AHA for Nascent Protein Synthesis (C10102)
	O-GlcNAc-modified glycoproteins	Click-iT™ GlcNAz Metabolic Glycoprotein Labeling Reagent (C33367)
	Sialic acid-modified glycoproteins	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)

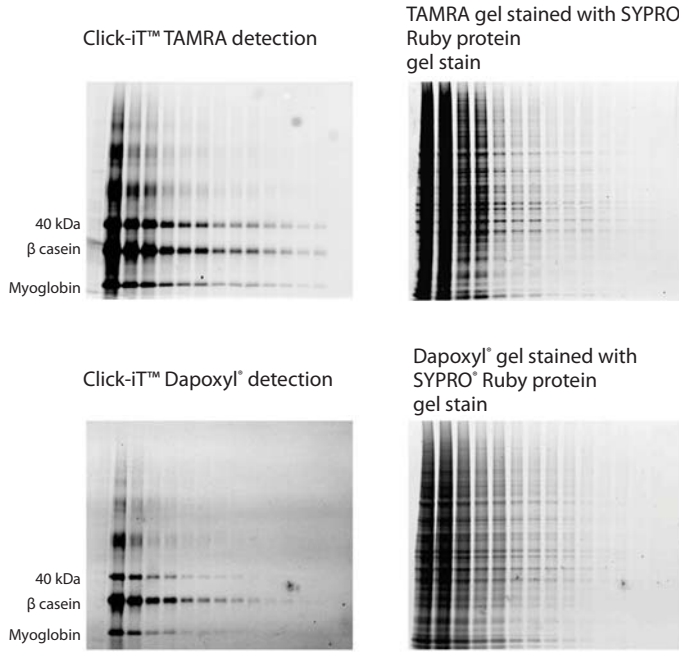

Figure 2. Sensitivity and multiplex compatibility of Click-iT™ Protein Analysis Detection Kits. Three model azido proteins (0.5 µg each of a 40 kDa model protein with single N-terminal azide, β-casein, and myoglobin) were spiked into 60 µg of Jurkat cell lysate and labeled with the Click-iT™ TAMRA alkyne or Dapoxyl® alkyne protein analysis detection kit, then precipitated and resolubilized in NuPAGE® LDS Sample Buffer. Two-fold serial dilutions (80 ng in lane 1) were separated by 1D PAGE on NuPAGE® Novex® 4–12% Bis-Tris gels. TAMRA labeled gels were imaged on the FX™ Pro Plus laser scanner (Bio-Rad) using 532 nm excitation and 555LP emission (upper left panel). Dapoxyl® labeled gels were imaged on the LumiImager (Boehringer Mannheim/Roche) at UV excitation and 600 ±20 nm emission (lower left panel). The gels were then fixed and post-stained with SYPRO® Ruby protein gel stain and imaged on the FX™ Pro Plus scanner using 488 nm excitation, 555LP emission (right panels). Detection sensitivity is 0.5–3 fmol for the TAMRA labeled proteins and 10–100 fmol for the Dapoxyl® labeled proteins.

Table 3. Detection and Multiplexed Proteomics® Compatibility of the Click-iT™ Protein Analysis Detection Kits.

Product	Cat. no.	Ex/Em*	Excitation Source	Detection Method	Multiplexed Proteomics® Compatibility
Click-iT™ Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit	C33370	545/580 nm	300 nm UV illumination or 532 nm laser	1D or 2D gel Western blot Mass spectrometry	• Pro-Q® Emerald 300 glycoprotein gel stain • SYPRO® Ruby protein gel stain • Western detection with anti-TAMRA antibody
Click-iT™ Dapoxyl® Protein Analysis Detection Kit	C33371	370/580 nm	300 or 365 nm UV illumination	1D or 2D gel Mass spectrometry	• Pro-Q® Diamond phosphoprotein gel stain • SYPRO® Ruby protein gel stain
Click-iT™ Biotin Protein Analysis Detection Kit	C33372	Not applicable	Not applicable	Western blot Mass spectrometry	Western detection with streptavidin

*Ex/Em = Excitation and emission maxima in nm.

Before You Begin

Materials Required but Not Provided

- 2–4 mg/mL azide-labeled protein sample in 1% SDS, 50 mM Tris-HCl, pH 8.0 prepared according to protocols described in MP33365 or MP33368
- 18 megaOhm water
- Methanol
- Chloroform

Experimental Protocols

Stock Solution Preparation

1.1 Add 60 µL of the alkyne solution (Component A) to the Click-iT™ Reaction Buffer (Component B). This solution may be stored at ≤–20˚C for up to 1 year.

1.2 Add 500 µL of 18 megaOhm water to the Click-iT™ Reaction Buffer Additive 2 (Component E). This solution may be stored at 2–6˚C for up to 6 months.

Labeling the Azide-Modified Proteins

2.1 Prepare Click-iT™ Reaction Buffer Additive 1 (Component D) **fresh** on the day of use. Add 100 µL of 18 megaOhm water to 1 vial. Store the solution at 2–6˚C for up to 1 week.

2.2 Add the following to a 1.5 mL microcentrifuge tube

- Up to 200 µg in a maximum volume of 50 µL of azide-labeled protein in 1% SDS, 50 mM Tris-HCl, pH 8.0 prepared according to protocols described in MP33365, MP33368, or MP10102.
- 100 µL of 2X Click-iT™ Reaction Buffer containing the alkyne detection reagent prepared in step 1.1.
- Sufficient volume of 18 megaOhm water for a final volume of 160 µL.

2.3 Cap the tube and vortex for 5 seconds.

2.4 Add 10 µL of $CuSO_4$ (Component C) and vortex for 5 seconds.

2.5 Add 10 µL of Click-iT™ Reaction Buffer Additive 1 (prepared in step 2.1) and vortex for 5 seconds. Wait for 2–3 minutes before proceeding to step 2.6.

2.6 Add 20 µL of reconstituted Click-iT™ Reaction Buffer Additive 2 (prepared in step 1.2) and vortex for 5 seconds. The solution turns bright orange.

2.7 Vortex continuously or rotate end-over-end for 20 minutes using a rotator. If using TAMRA or Dapoxyl® alkyne, cover the tube with foil to minimize light exposure.

Sample Preparation for 1D or 2D-PAGE Analysis

Note: If you used the Click-iT™ *O*-GlcNAc Enzymatic Labeling System (C33368), given the small quantity of protein, 20 µg, used for the α-crystallin positive control reaction, steps 3.1–3.8 are not recommended.

3.1 Add 600 µL of methanol to the reaction mixture and vortex briefly.

3.2 Add 150 µL of chloroform and vortex briefly.

3.3 Add 400 µL of 18 megaOhm water and vortex briefly.

3.4 Centrifuge for 5 minutes at 13,000–18,000 × g, then carefully remove and discard as much of the upper aqueous phase as possible while leaving the interface layer containing the protein precipitate intact. **Note:** The upper phase will be bright orange. The lower phase will be pink for the TAMRA reagent, and colorless for the Dapoxyl® and biotin reagents.

3.5 Add 450 µL of methanol to the tube and vortex briefly.

3.6 Centrifuge for 5 minutes at 13,000–18,000 × g to pellet the protein, then remove and discard the supernatant.

3.7 Add 450 µL of methanol to the tube and vortex briefly. Centrifuge and discard the supernatant. This second methanol wash step serves to remove residual reaction components.

3.8 Cover the tube with a lint-free tissue and keep the tube cap open. Allow the pellet to air-dry, for 15 minutes to overnight.

3.9 Cap the tube and store the sample at –20°C until use.

1D or 2D-PAGE Analysis

If a protein assay is desired to verify the sample concentration, the EZQ® Protein Quantitation Kit (Cat. no. R33200) is compatible with 1D and 2D sample loading buffers and requires only 1 µL of sample.

4.1 Resolubilize the precipitated sample (prepared in step 3.8) in 1D or 2D-PAGE sample loading buffer.

For 1D SDS gels, vortex the sample for 10 minutes followed by heating for 10 minutes at 70°C.

For 2D urea gels, vortex the sample for 10 minutes followed by heating for 10 minutes at 37°C.

Briefly spin the protein sample before loading to remove any unsolubilized material. Suggested loading amounts for 1D minigels are 10 µg for proteins labeled with the nascent protein synthesis labeling reagent and 20 µg for proteins labeled with one of the glycoprotein labeling reagents. A target concentration of approximately 1–2 mg/mL is suggested. Add the sample loading buffer to the α-crystallin positive control from the Click-iT™ O-GlcNAc Enzymatic

Labeling System (C33368). The α-crystallin concentration of this control is about 0.1 µg/µL before addition of sample loading buffer. Load 0.1–1.0 µg (5–50 pmol) on the gel or blot.

4.2 Perform electrophoresis.

4.3 Following electrophoresis, remove the gel from the cassette. Bis-Tris gels may be imaged immediately after removal from the cassette or after a short 5 minute water wash. If imaged immediately, use water to rinse off some of the SDS on the side of the gel that will be in contact with the imager tray. Add a little water to the imager tray before laying the gel down to prevent the gel from sticking. Tris-Glycine gels should be given a 5 minute water wash before imaging.

4.4 Image Click-iT™ labeled proteins (Table 2) directly if Dapoxyl® or TAMRA alkyne detection reagents were used. If biotin alkyne was used, perform a western transfer procedure.

4.5 After imaging gels of TAMRA- and Dapoxyl®-labeled samples can be fixed and then stained with Invitrogen's Multiplexed Proteomics™ technologies (Table 3).

Reference

1. Angew Chem Int Ed 41, 2596 (2002).

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
C33370	Click-iT™ Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit *UV/532 nm excitation* *10 reactions*	1 kit
C33371	Click-iT™ Dapoxyl® Protein Analysis Detection Kit *for UV excitation* *10 reactions*	1 kit
C33372	Click-iT™ Biotin Protein Analysis Detection Kit *10 reactions*	1 kit
Related Products		
A6397	anti-tetramethylrhodamine, rabbit IgG fraction *1 mg/mL*	0.5 mL
C10102	Click-iT™ AHA (L-azidohomoalanine) *for nascent protein synthesis*	5 mg
C21852	CandyCane™ glycoprotein molecular weight standards *200 gel lanes*	400 µL
C33365	Click-iT™ GalNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetylgalactosamine) *for *O*-linked glycoproteins* *5.2 mg*	1 each
C33366	Click-iT™ ManNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetyl-D-mannosamine) *for sialic acid glycoproteins* *5.2 mg*	1 each
C33367	Click-iT™ GlcNAz metabolic glycoprotein labeling reagent (tetraacetylated *N*-azidoacetylglucosamine) *for *O*-GlcNAc-modified proteins* *5.2 mg*	1 each
C33368	Click-iT™ *O*-GlcNAc Enzymatic Labeling System *for *N*- or *O*-linked GlcNAc glycoproteins* *10 labelings*	1 kit
C33373	Click-iT™ *O*-GlcNAc peptide and phosphopeptide LC/MS standards *5 nmol each*	1 set
C33374	Click-iT™ *O*-GlcNAc peptide LC/MS standard (H-Thr-Ala-Pro-Thr-(*O*-GlcNAc)Ser-Thr-Ile-Ala-Pro-Gly-OH) *Theoretical Mass (M+H): 1118.50*	5 nmol
M33305	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #1 *with 1 L each of Pro-Q® Diamond (P33300) and SYPRO® Ruby (S12000) gel stains*	1 set
M33306	Multiplexed Proteomics® Phosphoprotein Gel Stain Kit #2 *with 200 mL each of Pro-Q® Diamond (P33301) and SYPRO® Ruby (S12001) gel stains*	1 set
M33307	Multiplexed Proteomics® Glycoprotein Gel Stain Kit *with 1 L each of Pro-Q® Emerald 300 and SYPRO® Ruby (S12000) gel stains*	1 kit
P33350	PeppermintStick™ phosphoprotein molecular weight standards *200 gel lanes*	400 µL
P21855	Pro-Q® Emerald 300 Glycoprotein Gel Stain Kit *with SYPRO® Ruby protein gel stain* *10 minigels*	1 kit
P33300	Pro-Q® Diamond phosphoprotein gel stain	1 L
P33301	Pro-Q® Diamond phosphoprotein gel stain	200 mL
P33302	Pro-Q® Diamond phosphoprotein gel stain *bulk packaging*	5 L
R33200	EZQ® Protein Quantitation Kit *2000 assays*	1 kit
S12000	SYPRO® Ruby protein gel stain	1 L
S12001	SYPRO® Ruby protein gel stain	200 mL
S21900	SYPRO® Ruby protein gel stain *bulk packaging*	5 L

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