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Click-&-Go™ PC Protein Enrichment Kit
for capture and release of azide-modified proteins

Product No. 1150

Introduction

Photocleavable Click-&-Go™ Protein Enrichment Kit is an efficient, biotin/streptavidin-free tool for capturing azide-tagged biomolecules on a cleavable agarose resin and its subsequent selective, and reagent-free photorelease. The alkyne modification can occur via metabolic labeling, enzymatic addition, or chemical modification. Azide-modified proteins, or their post-translationally modified forms, are captured from complex protein extracts on the cleavable alkyne agarose resin supplied. Once covalently attached to the resin via copper catalyzed click chemistry, beads can be washed with high stringency virtually eliminating any non-specifically bound proteins. Irradiation with a hand-held UV-Vis lamp yields a highly enriched population of nascent proteins.

Kit Contents

Component	Amount	Storage
PC Alkyne-agarose resin, 50% slurry (Component A)	2 mL	2-4^{0}C
Lysis buffer (Component B)	7 mL	2-4^{0}C
Urea (Component C)	4.8 g	2-25^{0}C
Additive 1 (Component D)	1.5 mL	2-4^{0}C
Copper (II) Sulfate, 100 mM solution (Component E)	0.5 mL	2-25^{0}C
Additive 2 (Component F)	400 mg	2-25^{0}C
Agarose SDS wash buffer (Component G)	7.7 g	2-25^{0}C
Empty spin columns (Component H)	10	2-25^{0}C

Materials Required but Not Provided for Capturing of Alkyne-tagged Proteins

- 5-20 mg azide-tagged cell or tissue extract
- Unlabeled negative control cells or tissue
- Sample rotator
- Table top centrifuge
- Protease Inhibitor
- 2 mL microcentrifuge tubes
- 18 MΩ water, acetonitrile
- Hand-held UV lamp
- Probe sonicator

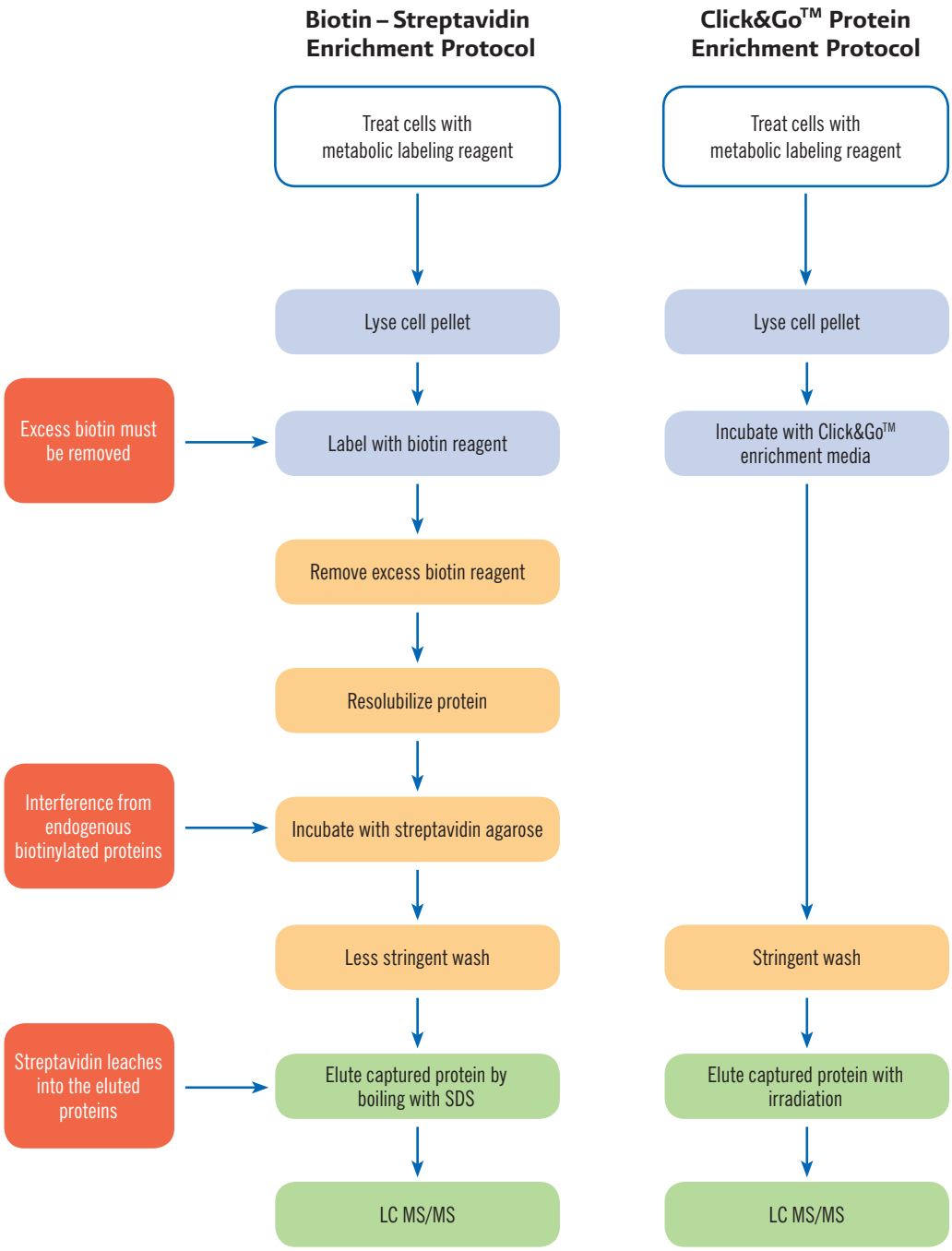

Figure 1. Schematic representation of pull–down workflows for biotin–streptavidin and Click&Go™ enrichment protocols.

Preparation of Stock Solutions

Lysis Buffer (200 mM Tris pH 8, 4% CHAPS, 1 M NaCl, 8 M Urea)

Add the entire bottle of **Solid Urea** (**Component C**) to the bottle containing **Lysis Buffer** (**Component B**) provided. Mix the solution on a rotator at room temperature until urea is completely dissolved (1-2 hours). Store refrigerated (for up to 1 week) or at -20^{0}C for 1 year to avoid decomposition of urea.

Note - 30 minutes before starting the enrichment protocol add 20 uL Protease Inhibitor Cocktail (e.g. Sigma 8340) for each 1 mL of Lysis buffer (sufficient for 50-200 million cells or 5-20 mg tissue extract) to be used.

Agarose SDS Wash Buffer (100 mM Tris, 1% SDS, 250 mM NaCl, 5 mM EDTA, pH 8.0)

Transfer the dry-blend powdered **Agarose SDS Wash Buffer** (**Component G**) mix in the aluminum packet into a beaker and add 190 mL 18 MΩ water, mix until all solids are dissolved then adjust pH of the solution to 8.0 ± 0.05 with 6N HCl. Bring the final volume to 200 mL with 18 MΩ water. Filter sterilize for long-term storage.

Click Reaction Additive 2

Add 2 mL of 18 MΩ water to **Additive 2** (**Component F**) and vortex until fully dissolved. After use, store remaining stock solution at -20C for up to 1 year.

Protein Enrichment Protocol (per enrichment)

Preparation of PC Alkyne-Agarose Resin (Step 1)

1.1 Mix the **50% resin slurry** (**Component A**) until the resin is completely resuspended.

1.2 Before the resin settles, transfer 200 µL of well-mixed resin with a 1 mL pipette into a clean 2 mL microfuge tube.

1.3 Add 1.3 mL 18 MΩ water to the resin.

1.4 Pellet the resin by centrifugation for 2 minutes at 1000 x g.

1.5 Carefully aspirate the supernatant leaving approximately 200 µL of settled resin at the bottom of the tube, take care not to aspirate settled resin.

Lysate Preparation (Step 2)

2.1 Add 1 mL **Lysis Buffer containing Protease Inhibitor** (see Preparation of Stock Solutions) to each azide-containing cell or tissue extract containing 5-20 mg protein in a 2 mL microfuge tube.

2.2 Incubate the lysis mixture on ice for 5-10 minutes.

2.3 While on ice, sonicate the mixture using a probe sonicator by applying two 3-second pulses. Take care not to overheat the sample.

2.4 Repeat step 2-3 until the lysate is no longer viscous (e.g. viscosity of water).

2.5 Centrifuge the lysate at 10,000 x g for 5 minutes.

2.6 Place lysate back on ice until ready for the click reaction.

Preparation of 2X Copper Catalyst Solution (Step 3)

3.1 Prepare 1 mL of 2X Copper Catalyst Solution per enrichment reaction as follows:

860 µL	18 MΩ water
100 µL	**Additive 1 (Component D)**

20 µL	**Copper (II) Sulfate Solution (Component E)**
20 µL	**Additive 2 (Component F)**

3.2 Vortex 2X Copper Catalyst Solution to mix.

Lysate/Agarose Click Reaction (Step 4)

4.1 Assemble the click reaction in a 2 mL microfuge tube as follows:

200 µL	washed PC Alkyne-Agarose resin (Step 1.5)
800 µL	cell or tissue lysate (Step 2.6)
1000 µL	2X Copper Catalyst Solution (Step 3.2)

4.2 Rotate end-over-end on sample rotator for 16-20 hours

Resin Wash (Step 5)

Agarose Wash Buffer w/SDS is used for stringent removal of non-specifically bound proteins. After this wash, it is critical to remove residual SDS by washing exhaustively with 8 M urea and 20% acetonitrile prior to mass spectrometry analysis.

5.1 Twist off the spin column's bottom closure and remove the cap.

5.2 Using a 1 mL pipette resuspend the resin from Step 4.2, then transfer the resin to a spin column.

5.3 Rinse the resin tube with 0.5 mL H_2O, and then transfer this volume to the spin column.

5.4 Centrifuge agarose resin for 1 minute at 1000 x g.

5.5 Add 2 mL of Agarose Wash Buffer W/SDS to the spin column, centrifuge at 1000 x g for 1 minute. Repeat this step 5 times.

5.6 Add 2 mL of 8 M urea/100 mM Tris pH 8 (not provided) to the spin column, centrifuge at 1000 x g for 1 minute. Repeat this step 5-10 times.

5.7 Add 2 mL of 20% acetonitrile (not provided) to the spin column, centrifuge at 1000 x g for 1 minute. Repeat this step 5–10 times.

Release of Resin-Bound Proteins (Step 6)

6.1 Resuspend the washed resin in 1 ml of PBS and transfer to a clear glass vial or quartz cuvette with a tight fitting cap.

6.2 Irradiate the resin suspension with light at 345-375 nm with constant agitation for 1 hour. This can be done using hand held long wave UV lamp such as UVGL-25.[1]

6.3 Agitate the sample at 37°C for 1 hour after irradiation. Avoid using a stir bar as this can crush some resins.

6.4 Collect the eluant by centrifugation or using an empty spin column.

[1] For more efficient photorelease and shorter irradiation time following UV-Lamps can be used:
http://uvp.com/3uvlamps.html
http://www.uvsystems.com/store/product.php?productid=16135&cat=250&page=1
http://www.uvsystems.com/store/product.php?productid=16193&cat=251&page=1

6.5 Resuspend the resin in 1 ml of PBS and agitate for 2-16 hours. For more efficient recovery of enriched protein(s), use a buffer containing 0.1-1% detergent and/or 250 mM – 1 M NaCl.

6.6 Collect the second elution by centrifugation or using an empty spin column.

Troubleshooting

Problem	Possible Cause	Solution
Low yield of enriched proteins	Inefficient protein capture or low abundance of alkyne-tagged proteins	Increase lysate concentration (use more cells) or pre-enrich the proteins (e.g. soluble lysate, membrane lysate, lectin enrichment, etc.). Confirm peptide recovery by measuring A280 after digestion
	Inefficient digestion of resin-bound proteins	Use high quality trypsin
High background with unlabeled control cells	Insufficient washing of resin	Increase column washes Use only high purity reagents Prepare filtered buffers fresh Ensure proper preparation of copper catalyst solution
Signal suppression during MS analysis	SDS contamination in digest	Wash the resin thoroughly after the Agarose Wash Buffer w/SDS wash with another buffer such as 8M urea and 20% acetonitrile to remove all traces of SDS detergent
Poor Photorelease	Light is not sufficiently intense	Use a lamp with a higher intensity.
	Incorrect wavelength of light	Ensure that the lamp is outputting light in the 345-368nm range.
	Insufficient agitation	Ensure that the beads are being properly mixed during photorelease
	Strong non-specific interactions	Consider using a detergent during photorelease or including more wash steps after photorelease