DATASHEET

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Click-&-Go™ Protein Enrichment Kit
for capture of alkyne-modified proteins

Product Number: CCR-1039

Introduction

The Click-&-Go™ Protein Enrichment Kit is an efficient , biotin-streptavidin free tool for capture of alkyne-modified proteins on an azide-agarose resin. The alkyne modification can occur via metabolic feeding, anzymatic addition, or chemical modification. Alkyne-modified proteins, or their post-translationally modified forms, are captured from complex protein extracts on the azide-agarose resin supplied. Once covalently attached to the resin via copper catalysed click chemistry, beads can be washed with 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 M/MS) analysis.

Kit Contents

Component	Amount	Storage
Azide –agarose resin, 50% slurry (Component A)	2ml	2-4°C
Lysis Buffer (Component B)	7ml	2-4°C
Urea (Component C)	4.8g	2-25°C
Additive 1 (Component D)	1.5ml	2-4°C
Copper (II) Sulphate, 100mM solution (Component E)	0.5ml	2-25°C
Additive 2 (Component F)	400mg	2-25°C
Agarose SDS Wash Buffer (Component G)	7.7g	2-25°C
Empty Spin Columns (Component H)	10	2-25°C

Materials Required but not Provided

- 5-20mg alkyne-tagged cell or tissue extract
- unlabelled negative control cells or tissue
- sample rotator
- table-top centrifuge
- protease inhibitor
- 2ml microcentrifuge tubes
- 18MΩ water
- probe sonicator

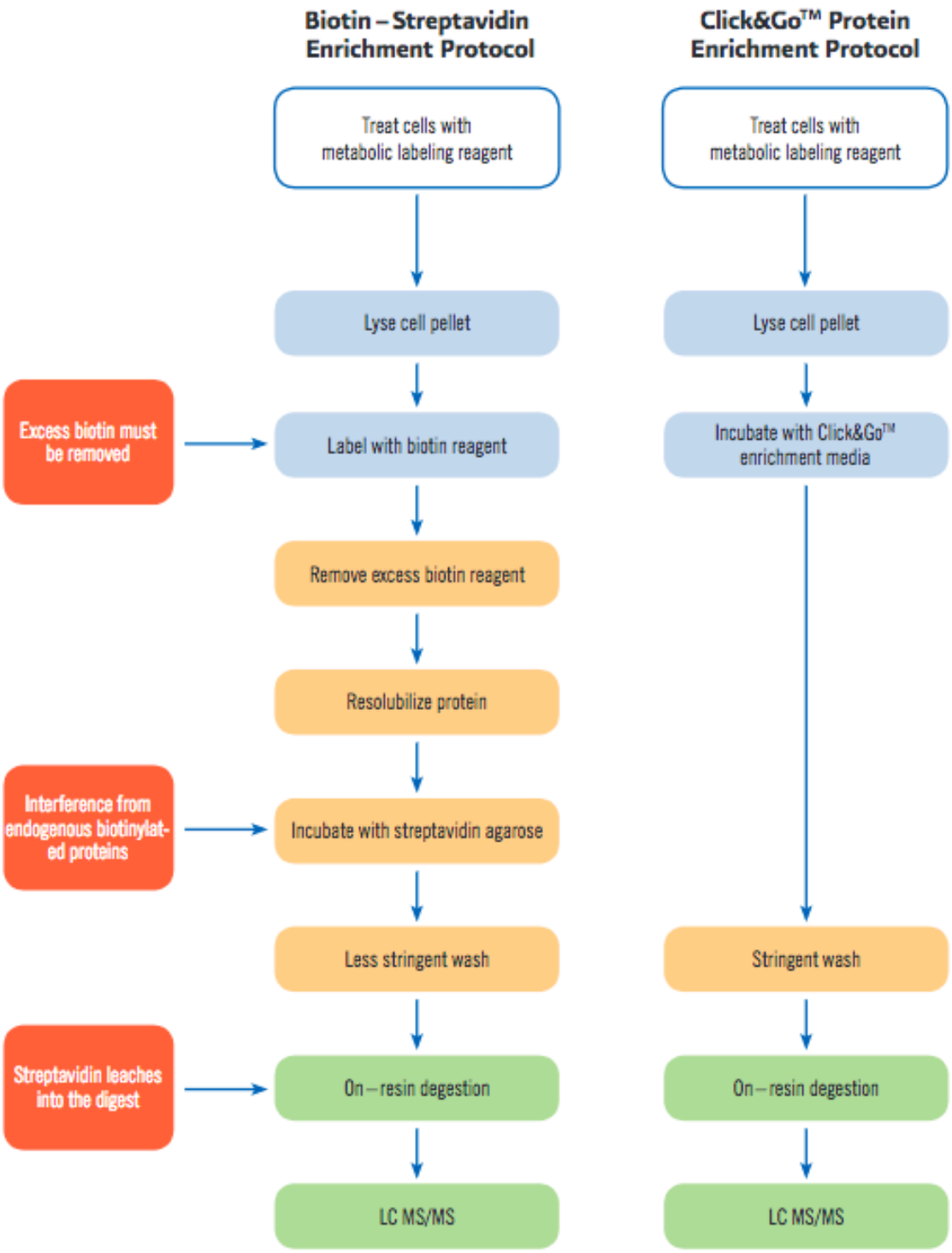

Figure 1. Schematic representation of pull – down workflows for biotin – streptavidin and Click&Go™ enrichment protocols.

Preparation of Stock Solutions

Lysis Buffer (200mM Tris pH8, 4% CHAPS, 1M NaCl, 8M 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°C for 1 year to avoid decomposition of urea.

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

Agarose SDS Wash Buffer (100mM tris, 1% SDS, 250mM NaCl, 5mM EDTA, pH8)

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

Click reaction Additive 2

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

Protein Enrichment Protocol (per enrichment)

Preparation of Azide-Agarose Resin (Step 1)

1. Mix the **50% Resin Slurry (Component A)** until the resin is completely resuspended.
2. Before the resin settles, transfer 200µl of well-mixed resin with a 1ml pipette into a clean 2ml microfuge tube.
3. Add 1.3ml 18MΩ water to the resin.
4. Pellet the resin by centrifugation for 2 minutes at 1000xg.
5. Carefully aspirate the supernatant leaving approximately 200µl of settled resin at the bottom of the tube, take care not to aspirate settled resin.

Lysate Prepatation (Step 2)

1. Add 1ml **Lysis Buffer containing Protease Inhibitor** (see Preparation of Stock Solutions) to each azide containing cell of tissue extract containing 5-20mg protein in a 2ml microfuge tube.
2. Incubate the lysis mixture on ice for 5-10 minutes.
3. While on ice, sonicate the mixture using a probe sonicator by applying two 3-second pulses. Take care not to overheat the sample.
4. Repeat step 2-3 until the lysate is no longer viscous (e.g. viscosity of water).
5. Centrifuge the lysate at 10,000xg for 5 minutes.
6. Place lysate back on ice until ready for the click reaction.

Preparation of 2X Copper Catalyst Solution (Step 3)

1. Prepare 1ml of 2X Copper Catalyst Solution per enrichment reaction as follows:
   860µl 18MΩ water
   100µl **Additive 1 (Component D)**
   20µl **Copper (II) Sulphate Solution (Component E)**
   20µl **Additive 2 (Component F)**
2. Vortex 2X Copper Catalyst Solution to mix

Lysate/Agarose Click Reaction (Step 4)

1. Assemble the click reaction in a 2ml microfuge tube as follows:
   200µl washed Azide-Agarose resin (Step 1.5)
   800µl cell or tissue lysate (Step 2.6)
   1000µl 2X Copper Catalyst Solution (Step 3.2)
2. Rotate end-over-end on sample rotator for 16-20 hours

Reduction and Alkylation of Resin Bound Proteins (Step 5)

1. Warm Agarose Wash Buffer w/SDS to room temperature before starting, making sure the solution is homogeneous and clear before use.
2. Centrifuge agarose resin (Step 4.2) for 1 minute at 1000xg. Aspirate the supernatant to waste, taking care not to aspirate the resin.
3. Add 1.8ml of 18MΩ water to the resin, centrifuge at 1000xg, aspirate the supernatant to waste taking care not to aspirate resin. This water wash step prevents clumping of the resin caused by interation of residual Lysis Buffer with the SDS on Agarose Wash Buffer.
4. Add 1ml Agarose Wash Buffer w/SDS and 10µl of 1M DTT to the resin. Vortex briefly to resuspend the resin.
5. Heat the resin at 70°C on a heat block for 15 minutes, then cool to room temperature for 15-30minutes.
6. Centrifuge resin for 5 minutes at 1000xg, aspirate the supernatant to waste taking care not to aspirate the resin.
7. Prepare 1ml of a 40mM iodoacetamide solution per enrichment reaction by dissolving 7.4mg of iodoacetamide into 1ml of Agarose Wash Buffer w/SDS.
8. Add 1ml 40mM iodoacetamide solution to the resin, vortex to resuspend the resin, incubate the reaction in the dark for 30 minutes at room temperature.

Resin Wash (Step 6)

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 8M Urea and s0% acetonitrile propr to mass spectrometry analysis.

1. Twist off the spin column's bottom closure and remove the cap.
2. Using a 1ml pipette resuspend the resin from Step5.8, the transfer the resin to a spin column.
3. Rinse the resin rube with 0.5ml H_2O, and the also transfer this volume to the spin column.
4. Add 2ml of Agarose Wash Buffer w/SDS to the spin column, centrifuge at 1000xg for 1 minute. Repeat this step 5 times.
5. Add 2ml of 8M Urea/100mM Tris pH8 (not provided) to the spin column, centrifuge at 1000xg for 1 minute. Repeat this step 5-10 times.
6. Add 2ml of 20% acetonitrile (not provided) to the spin column, centrifuge at 1000xg for 1 minute. Repeat this step 5-10 times.

Protease Digestion of Resin-Bound Proteins (Step 7)

1. Cap the bottom of the spin column, add 500µl of digestion buffer (100mM tris, 2mM $CaCl_2$, 10% acetonitrile (not provided) to the resin.
2. Using a 1ml pipette to resuspend the resin in the spin column, then transfer the resin to a clean tube.
3. Rinse the spin column with 0.5ml additional digestion buffer, and then add this rinse to the transferred resin.
4. Pellet the resin by centrifugation for 5 minutes at 1000xg. Aspirate the supernatant to waste, leaving approximately 200µl of digestion buffer in the tube above the resin, taking care not to aspirate the resin.
5. Add 10µl of 0.1µg/µl trypsin to he resin slurry, gently mix the slurry, then incubate at 37°C for 6 hours or overnight.

Preparation of Digest for Mass Spectrometry Analysis

1. Pellet the resin from Step 7.5 by centrifugation for 5 minutes at 1000xg, then carefully transfer the digested supernatant to a clean tube.
2. Add 500µl 18MΩ water to the resin. Vortex briefly to mix then pellet the resin by centrifugation for 5 minutes at 1000xg.
3. Transfer the rinse supernatant over the resin to the digest supernatant from step 8.1.
4. Add additional 18MΩ water to the digest to a final volume of 1ml (note this dilutes the acetonitrile concentration to 2%).
5. Acidify the diluted digest by adding 2µl of TFA.
6. Desalt the digest on a C-18 cartridge using a vacuum or gravity flow, allowing each solution to completely flow through the cartridge before adding the next solution.
    a. Add 1ml of 50% acetonitrile/0.1% TFA to the cartridge and discard the effluent.
    b. Add 1ml of 0.1% TFA to the cartridge and discard the effluent. Repeat one more time.
    c. Add the acidified, diluted digest to the cartridge and discard the effluent.
    d. Add 1ml of 0.1% TFA to the cartridge and discard the effluent. Repeat one more time.
    e. Place a clean 1.5ml tube below the C-18 cartridge outlet.
    f. Elute the peptides into a clean 1.5ml tube by adding 700µl of 50% acetonitrile/0.1% TFA to the C-18 cartridge.
7. Dry the eluate containing the desalted peptide digest in a vacuum concentrator. Store at -20°C until ready for MS analysis.

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 protein	Use high quality trypsin
High background with unlabelled 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.