

Copper-click Alkyne / Azide Condensation Reaction
with peptide or tentagel beads

Notes: keep fluors in dark, dry DMSO dry.

Materials

for 1 reaction:

-2uL 2 mM label, containing azide or alkyne, in DMF or DMSO

-50uL peptide or bead sample containing complementary azide or alkyne.

Up to 40 uM concentration (for 2:1 label:macromolecule) in 50 mM Tris pH 8

-Click-iT reaction buffer components stored in click-it box in -20C freezer:

-100 uL Component A:

-10 uL Component B: CuSO₄, treat as hazardous waste.

-10 uL additive 1: Component C + water. 100uL aliquots stored, use open one.

Additive 1 contains a reducing agent that can oxidize. Be aware of color change, additive turns from colorless to brown.

-20 uL additive 2: component D + water.

Protocol

- I. Put 98 uL component A in a 1.5 ml tube
- II. Add 2uL label, for a final concentration of 40uM. Total amount: 4 nmole label.
- III. Add 50uL of peptide or bead in 50 mM Tris pH 8, cap tube, vortex ~ 5 seconds
- IV. Add 10uL of CuSO₄ (component B), then vortex ~ 5 seconds.
- V. Add 10uL of additive 1, vortex ~ 5 secs.
Wait for 2-3 mins. but no longer than 5 mins, before the next step.
- VI. Add 20uL of additive 2, vortex ~ 5 secs.
Solution color change will occur, generating a bright orange color.
- VII. Using the rotisserie, rotate end over end for 20 mins using a rotator.
If light sensitive, protect sample from light with foil paper wrapped around tube.
- VIII. Remove products from reaction ASAP, filter and wash beads, freeze peptide at -80 or inject on HPLC