Bangs Laboratories
BEADS ABOVE THE REST

Click Chemistry Microspheres

Product Data Sheet 920

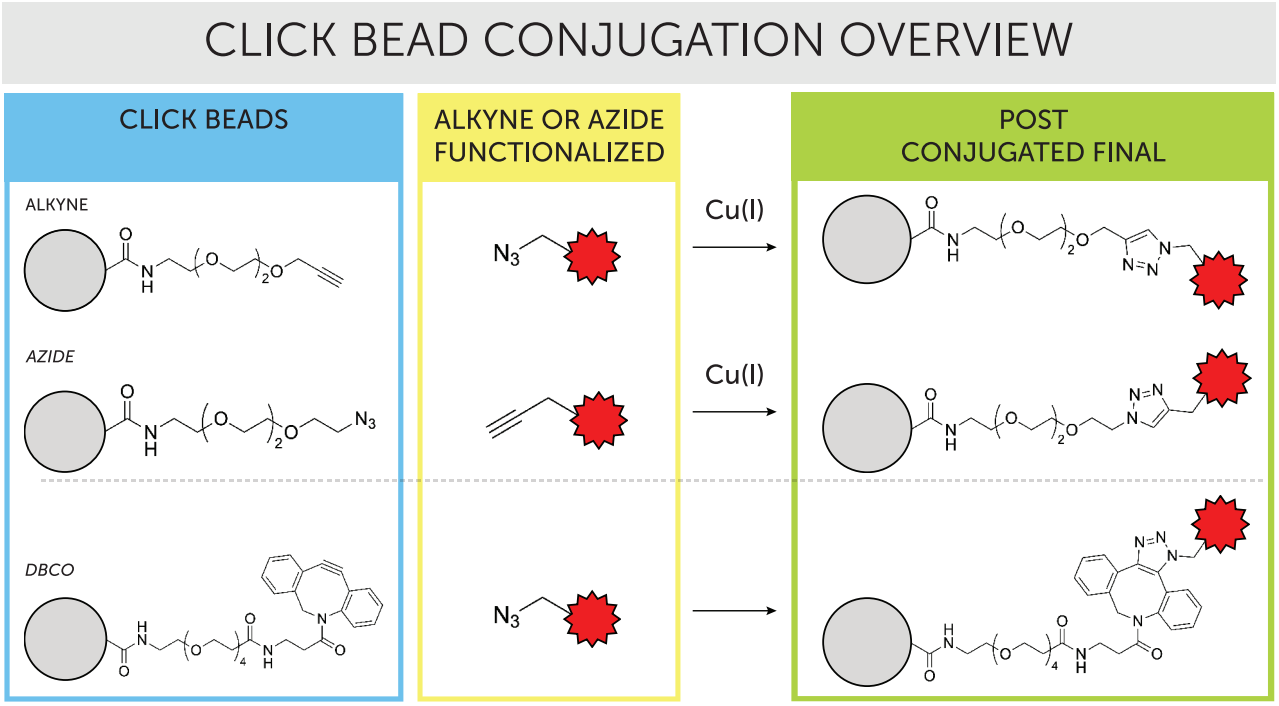
INTRODUCTION

Bangs Laboratories provides a variety of avenues to coat particles with your molecules of interest through the application of adsorption, covalent conjugation, and affinity binding techniques. While these procedures are reliable and versatile, there are limitations to their application based on the molecule of interest as certain functional groups must be accessible for a successful conjugation using traditional EDAC chemistry. We are pleased to announce we have expanded our conjugation capabilities by offering a Click-Chemistry class of particles.

DESCRIPTION

Click Chemistry (CC) exploits the CuAAC—Cu catalyzed alkyne azide cycloaddition reaction—to link separate molecules together to form a covalent 1,5-disubstituted 1,2,3-triazole. CC is employed in organic synthesis as well as biological reactions. Applications of CC include drug discovery, labeling biomolecules, and modifying nucleic acids, as well as many others.

Advantages of CC are its quick + selective aqueous reaction that is resilient in pH changes. The hands-on time is minimal since a simple modified alkyne, azide, or strained alkyne present on your molecule of interest (MOI) allows for attachment to one of our CC particles. We offer beads coated with strained alkynes for applications requiring copper-free conditions, along with azide and alkyne functional groups for more tolerant applications.



Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) for coupling of azide and alkyne-functionalized particles

The basic procedure below describes the ligation of a functionalized MOI to an appropiately functionalized Click Bead. Because this protocol uses either the MOI or Click Bead with an azide functional group, any buffers containing sodium azide (NaN_3) will interfere with the reaction.

EQUIPMENT

- Analytical balance
- Volumetric pipettes
- Disposable pipette tips
- Vortex mixer
- Pasteur pipettes and pipette bulb
- Centrifuge
- Magnetic separator
- End-over-end mixer and appropriately-sized bottles

MATERIALS

1. 1 mg/mL Copper Sulfate Solution:
    - Add 10 mg of Copper Sulfate Pentahydrate to a 15 mL centrifuge tube
    - Add 10 mL of DI water and vortex until copper has dissolved.
    - Store at 4 °C for up to one month.
    - General target $CuSO_4$ molar ratio: ~25% of functional groups on beads
        i. **Note:** The copper solution should be light blue in color. If the color has changed or any red precipitate has formed, the solution has decomposed and a fresh solution should be made.

2. 1 mg/mL THPTA Ligand Solution:
    - Add 10 mg of THPTA ligand to a 15 mL centrifuge tube.
    - Add 10 mL of DI water and vortex until THPTA has fully dissolved.
    - Store at 4 °C for up to one month.
    - General target THPTA ligand molar ratio: 4:1 ligand to copper ratio
        i. **Note:** The ligand solution should be colorless. If the solution appears off-color or any precipitate has formed, the solution has decomposed and a fresh solution should be made.

3. 1 mg/mL Sodium Ascorbate (NaAsc) Solution:
    - Add 1 mg of NaAsc to a 1.5 mL centrifuge tube.
    - Add 1 mL of DI water and vortex until NaAsc has fully dissolved.
    - General target NaAsc ligand molar ratio: 4:1 ligand to copper ratio
        i. **Note:** The NaAsc solution will oxidize over time and so this solution must be made the day it is to be used.

4. 0.2-1.0 g of Bangs Click Chemistry particles

5. Prepare a 1 mg/mL MOI-alkyne (Molecule Of Interest-alkyne) solution if using azide-coated Click Beads or MOI-azide solution if using alkyne-coated beads.

PROCEDURE

1. Pipette 0.5 mL of the particles to be assayed in a 1.5 mL centrifuge tube.
2. Add the calculated volumes of copper sulfate, THPTA ligand, and MOI stock solutions and briefly vortex to mix.
3. Add the calculated volume of the NaAsc solution to the bead mixture, cap the centrifuge tube, and briefly vortex.
    i. **Note:** Once the NaAsc has been added, the centrifuge tube should not be allowed to remain open to the air for any prolonged periods of time. This will cause the copper catalyst to oxidize and can cause the reaction/assay to fail.
4. Cover the centrifuge tube in a piece of aluminum foil to keep it away from stray light, and allow the tube to roll end-over-end for at least 4 hours. If the assay is set up at the end of the day, the reaction can roll over night without issue.
5. After the prescribed reaction time, settle the beads by either placing them on a magnetic separator or by spinning them at 9000 RPM for 5 minutes in a microcentrifuge.
6. Remove supernatant.
7. Reconstitute in water to the desired concentration.

Strain-Promoted Alkyne-Azide Cycloaddition (SPAAC) for coupling of alkyne-functionalized particles

This protocol is for biological applications where copper has a deleterious effect. The particle is functionalized with alkyne only; therefore, an MOI-azide is required to react with the particle. Because your MOI has an azide functional group, any buffers containing sodium azide (NaN_3) will interfere with the reaction. This protocol only discusses the reaction. For preparing cells or other biologics to express specific moieties, please refer to the citations at the end of this document.

EQUIPMENT

- Analytical balance
- Volumetric pipettes
- Disposable pipette tips
- Vortex mixer
- Pasteur pipettes and pipette bulb
- Centrifuge
- Magnetic separator
- End-over-end mixer and appropriately-sized bottles

MATERIALS

Prepare a 1 mg/mL MOI-azide (Molecule Of Interest-azide) solution

PROCEDURE

1. If microspheres were not stored on the roller, allow the material to roll for at least 30 minutes at room temperature.
2. Perform a microscopic examination of the microspheres to determine if any aggregation is present. If gross aggregation is present, the material is to be rejected for assaying. If minor aggregation Is observed, briefly sonicate the material in a bath sonicator and recheck. Repeat this process as needed until the particles are acceptable.
3. Pipette 0.5 mL of the particles to be assayed in a 1.5 mL centrifuge tube.
4. Add the calculated volume of MOI-azide stock solution to the beads and briefly vortex to mix.

PROCEDURE

5. Cover the centrifuge tube in a piece of aluminum foil to keep it away from stray light, and allow the tube to roll end-over-end for at least 4 hours. If the assay is set up at the end of the day, the reaction can roll over night without issue.
6. After the prescribed reaction time, settle the beads by either placing them on a magnetic separator or by spinning them at 9000 RPM for 5 minutes in a microcentrifuge.
7. Remove supernatant.
8. Reconstitute in water to the desired concentration.

REFERENCES

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ORDERING INFORMATION

Cat. Number	Product
CBPC001	Click Beads, PS-Azide, Low - 5.0µm
CBPC002	Click Beads, PS-Alkyne, Low - 5.0µm
CBPC003	Click Beads, PS-DBCO, Low - 5.0µm
CBMFY01a	Click Beads, Magnefy™ Azide, Low - 1.0µm
CBMFY01b	Click Beads, Magnefy™ Azide, Medium - 1.0µm
CBMFY01c	Click Beads, Magnefy™ Azide, High - 1.0µm
CBMFY02a	Click Beads, Magnefy™ Alkyne, Low - 1.0µm
CBMFY02b	Click Beads, Magnefy™ Alkyne, Medium - 1.0µm
CBMFY02c	Click Beads, Magnefy™ Alkyne, High - 1.0µm
CBMFY03a	Click Beads, Magnefy™ DBCO, Low - 1.0µm
CBMFY03b	Click Beads, Magnefy™ DBCO, Medium - 1.0µm
CBMFY03c	Click Beads, Magnefy™ DBCO, High - 1.0µm
CBPMC01a	Click Beads, ProMag® Azide, Low - 3.0µm
CBPMC01b	Click Beads, ProMag® Azide, Medium - 3.0µm
CBPMC01c	Click Beads, ProMag® Azide, High - 3.0µm
CBPMC02a	Click Beads, ProMag® Alkyne, Low - 3.0µm
CBPMC02b	Click Beads, ProMag® Alkyne, Medium - 3.0µm
CBPMC02c	Click Beads, ProMag® Alkyne, High - 3.0µm
CBPMC03a	Click Beads, ProMag® DBCO, Low - 3.0µm
CBPMC03b	Click Beads, ProMag® DBCO, Medium - 3.0µm
CBPMC03c	Click Beads, ProMag® DBCO, High - 3.0µm

Products are supplied at 1% solids.
Funtional group concentration: Low - 10µmol/g; Medium - 50µmol/g; High - 100µmol/g

Order online anytime at **www.bangslabs.com.**

TRADEMARKS AND REGISTERED TRADEMARKS

1. Magnefy™ - Bangs Laboratories, Inc.
2. ProMag® - Polysciences, Inc.