



CuAAC Cell Reaction Buffer Kit (THPTA based)

Cat. No.	Amount
CLK-074	1 kit

For research use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at 4 °C

Short term exposure (up to 1 week cumulative) to ambient temperature possible.

Shelf Life: 12 months after date of delivery

Description:

The CuAAC Cell Reaction Buffer Kit (THPTA based) is suitable to perform Copper (Cu(I))-catalyzed Azide-Alkyne Click chemistry reactions (CuAAC) with cells containing metabolically functionalized Alkyne- or Azide modified biomolecules.

1 Kit provides sufficient amounts to perform **50 CuAAC experiments à 500 µl using 2 mM CuSO₄ (copper source), 10 mM THPTA (Cu(I)-stabilizing ligand) and 100 mM Na-Ascorbate (reduction reagent)** in 100 mM Na-Phosphate reaction buffer.

Content:

Copper source:

2 x 10 mg CuSO₄ (M = 159.6 g/mol), #CLK-MI004)

Cu(I) stabilizing ligand:

5 x 25 mg THPTA (M= 434.5 g/mol, #CLK-1010)

Reduction Reagent:

4 x 200 mg Na-Ascorbate (M = 198.1 g/mol, #CLK-MI005)

Reaction Buffer:

2 x 30 ml sterile 100 mM Na-Phosphate Buffer, pH 7

10 ml sterile ddH₂O

Materials required but not provided:

Alkyne- or Azide-functionalized substrates e.g. fixed and permeabilized cells containing metabolically functionalized Alkyne- or Azide-modified biomolecules.

(Picoyl)-Azide or Alkyne detection reagent and appropriate solvent (e.g. DMSO)

For labeling of fixed and permeabilized cells:

Washing solutions e.g. PBS containing 3% BSA

Fixation solution e.g. PBS containing 3.7% formaldehyd

Permeabilization solution e.g. PBS containing 0.5% Triton X-100

Mounting medium for imaging

Additional labeling reagent such as nuclear stain or antibody

1. Introduction

Copper (Cu(I))-catalyzed **Azide-Alkyne Click** chemistry reactions (**CuAAC**) describe the reaction of an Azide-functionalized molecule A with a terminal Alkyne-functionalized molecule B that results in a stable conjugate A-B via a Triazole moiety.

Since terminal Alkynes are fairly unreactive towards Azides, the efficiency of CuAAC reactions strongly depends on the presence of a metal catalyst such as copper ions in the +1 oxidation state (Cu(I)).



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Different copper sources, reduction reagents and Cu(I) stabilizing ligands are available however, for most bioconjugation applications the combination of the Cu(II) salt CuSO_4 as copper source, a water-soluble Cu(I) stabilizing ligand such as BTAA and sodium ascorbate as a reduction reagent is recommended.^[1-3]

The use of Picolyl-Azide reagents instead of conventional Azide reagents can further increase the reaction efficiency and decrease the required final CuSO_4 concentration due to the internal copper chelating moiety.^[4]

The set-up of a CuAAC reaction is based on the following general three-step procedure:

- Prepare a mix of Alkyne- and Azide functionalized molecules in an appropriate reaction buffer.
- Prepare a CuSO_4 : Cu(I)-ligand premix, add it to the Azide- Alkyne solution and mix briefly.
- Add Na-Ascorbate as reduction reagent at last to start the reaction.

The CuAAC Cell Reaction Buffer Kit (THPTA based) provides sufficient amounts to perform 50 CuAAC experiments à 500 μl using 2 mM CuSO_4 , 10 mM THPTA and 100 mM Na-Ascorbate in 100 mM Na-Phosphate reaction buffer.

A general protocol for labeling of fixed and permeabilized cells containing metabolically functionalized Alkyne- or Azide-modified biomolecules is outlined below (see 3.) however, individual optimization might be required for different CuAAC labeling experiments as well as for critical reaction parameter e.g. final CuSO_4 concentration, CuSO_4 :THPTA ratio, detection reagent concentration.

Hong *et al.*^[2] and Presolski *et al.*^[1] provide useful background information on the influence of CuSO_4 concentration, CuSO_4 : ligand ratio and reaction buffer type that may be used as a starting point if optimization is required.

2. Preparation of stock solutions

The concentration of stock solutions (2.1 to 2.3) is suitable to prepare 500 μl assays containing 2 mM CuSO_4 , 10 mM THPTA and 100 mM Na-Ascorbate (see 3.3 and 3.4, respectively). Adjustments might be required if different assay volumes or final compound concentrations are used.

2.1 THPTA stock solution (Cu(I) stabilizing ligand)

- Add an appropriate amount of ddH₂O (Tab. 1), vortex until the compound is completely dissolved and spin down briefly.
- Prepare aliquots to avoid repeated freeze-thaw-cycles and store at -20 °C.

- The solution is stable up to 1 year at -20 °C.

Table 1 Volume of ddH₂O required for a 250 mM THPTA stock solution.

THPTA	Concentration of stock solution	Amount of ddH ₂ O
25 mg	250 mM	230 μl

2.2 CuSO_4 stock solution (copper source)

- Add an appropriate amount of ddH₂O (Tab. 2), vortex until the compound is completely dissolved and spin down briefly.
- Prepare aliquots to avoid repeated freeze-thaw-cycles and store at -20 °C.
- The solution is stable up to 1 year at -20 °C.

Table 2 Volume of ddH₂O required for a 100 mM CuSO_4 stock solution.

CuSO_4	Concentration of stock solution	Amount of ddH ₂ O
10 mg	100 mM	628 μl

2.3 Na-Ascorbate stock solution (reduction reagent)

- Add an appropriate amount of ddH₂O (Tab. 3), vortex until the compound is completely dissolved and spin down briefly.
- Prepare aliquots to avoid repeated freeze-thaw-cycles and store at -20 °C.
- The solution is stable up to 1 year at -20 °C.

Please note: Do not use solutions that appear brown. Freshly prepared, fully functional Na-Ascorbate solutions are colorless and turn brown upon oxidization thereby losing their reduction capability.

Table 3 Volume of ddH₂O required for a 1 M Na-Ascorbate stock solution.

Na-Ascorbate	Concentration of stock solution	Amount of ddH ₂ O
200 mg	1 M	1010 μl



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2.4 (Picoly)-Azide detection reagent stock solution

- (Picoly)-Azide detection reagents are not provided within this kit.
- Add an appropriate amount of suitable solvent e.g. DMSO to achieve a stock solution concentration of 10 mM for Azide detection reagents and 500 μ M for Picoly-Azide detection reagents.
- Final concentrations of Azide or Alkyne detection reagents may range from 2 μ M to 100 μ M. Final concentrations of Picoly-Azide detection reagents may range from 0.5 to 5 μ M (see 3.4).

3. General protocol for CLICK labeling of fixated and permeabilized cells containing metabolically functionalized Alkyne- or Azide-modified biomolecules

The protocol below is intended as a general guideline however, individual optimization might be required.

3.1 Metabolically label cells with an Alkyne or Azide-functionalized substrate

- Cultivate cells on coverslips under conditions that ensure optimal growth of cell type.
- Add Alkyne- or Azide-functionalized substrate at the desired final concentration and cultivate for an appropriate time under conditions optimal for metabolic incorporation of the modified substrate.

3.2 Fixate and permeabilize cells

Please note: The fixation with 3.7% formaldehyde in PBS and subsequent permeabilization with 0.5% Triton X-100 is a general guideline. Optimization might be required. Different reagent concentrations, different fixation and permeabilization reagents (e.g. methanol or saponin) or TBS as buffer solution instead of PBS can be used as well. Permeabilization is not required for cell surface or lipid component labeling.

- Remove cultivation medium
- Transfer each coverslip to a well of a 6-well plate
- Add 1 ml of 3.7% formaldehyde in PBS for fixation and incubate for 15 min. at room temperature.
- Remove fixation reagent and wash 2-3 times with PBS containing 3% BSA.
- Add 1 ml of Triton X-100 in PBS and incubate for 20 minutes at room temperature for permeabilization.

3.3 Prepare CuSO₄:THPTA-Premix

Please note: Both the final CuSO₄ concentration as well as CuSO₄:THPTA ratio are critical parameters for CuAAC reaction efficiency. A final CuSO₄ concentration of 2 mM and a CuSO₄:THPTA ratio of 1:5 is recommended as a starting point for labeling of fixed and permeabilized cells containing metabolically Azide- or Alkyne-functionalized biomolecules. Individual optimization for each assay is strongly recommended. Minimum CuSO₄ concentration: 50 μ M.

- Prepare the CuSO₄:THPTA-Premix freshly for each experiment.
- Allow all solutions to warm up to room temperature.
- Mix the appropriate amount of 100 mM CuSO₄ and 250 mM THPTA stock solution (Tab. 4) by vortexing and spin down briefly.
- 30 μ l CuSO₄:THPTA-Premix (1 Assay) is sufficient for the preparation of 500 μ l CLICK reaction cocktail (see. 3.4).

Table 4 Pipetting scheme for CuSO₄:THPTA-Premix (ratio 1:5).

Compound	Final conc.	1 Assay	10 Assays
100 mM CuSO ₄ stock solution (see 2.2)	33.33 mM	10 μ l	100 μ l
250 mM THPTA stock solution (see 2.1)	166.66 mM	20 μ l	200 μ l

3.4 Prepare CLICK reaction cocktail

Please note: Prepare CLICK reaction cocktail **freshly for each experiment** and **use it immediately but definitely within 15 minutes** after preparation. 500 μ l CLICK reaction cocktail (1 Assay) is sufficient to label one 18x18 coverslip.

- Allow all solutions to warm up to room temperature.
- Refer to Tab. 5 for appropriate amounts of compound stock solutions.
- 500 μ l CLICK reaction cocktail (1 Assay) is sufficient for a 18x18 coverslip.
- Add an appropriate amount of Azide- or Alkyne detection reagent solution to the reaction buffer, vortex and spin-down briefly.
- Add CuSO₄/THPTA Premix, vortex and spin down briefly.
- Add Na-Ascorbate, vortex and spin down briefly.



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Table 5 Pipetting scheme for CLICK reaction cocktail. Please add the compounds exactly in the order described below.

Compound	Final conc.	1 Assay (500 μ l)	10 Assays (5 ml)
100 mM Na-Phosphate reaction buffer, pH 7		419 μ l	4.19 ml
10 mM Azide or-Alkyne detection reagent stock solution (not provided, see 2.4)	20 μ M ^[1]	1 μ l	10 μ l
33.33 mM / 166.66 mM CuSO ₄ :THPTA-Premix (see 3.3)	2 mM / 10 mM	30 μ l	300 μ l
1 M Na-Ascorbate stock solution (see 2.3)	100 mM	50 μ l	500 μ l

^[1]Final concentrations of Azide or Alkyne detection reagents may range from 2 μ M to 100 μ M. Final concentrations of Picolyl-Azide detection reagents may range from 0.5 to 5 μ M. We recommend starting with 20 μ M or 5 μ M, respectively. Concentrations can be titrated down in case of high background or up in case of low signal.

3.5 Perform CLICK labeling of fixated and permeabilized Alkyne- or Azide-labeled cells

- Remove the permeabilization buffer (see 3.2) and wash 2-3 times with PBS containing 3% BSA.
- Add 500 μ l CLICK reaction cocktail (see 3.4) to each well containing one coverslip. Ensure that the coverslip is entirely covered with solution.
- Incubate samples 30 – 60 min at room temperature (protected from light).
- Remove CLICK reaction cocktail and wash cells 1-2 times with PBS containing 3% BSA.
- Remove wash solution.
- For nuclear staining with DAPI or Hoechst 33342 or antibody labeling wash once with PBS, remove PBS and proceed with staining according to the manufacturer's protocol.

Selected References:

- [1] Presolski *et al.* (2011) Copper-Catalyzed Azide-Alkyne Click Chemistry for Bioconjugation. *Current Protocols in Chemical Biology* **3**:153.
 [2] Hong *et al.* (2011) Analysis and Optimization of Copper-Catalyzed Azide-Alkyne Cycloaddition for Bioconjugation. *Angew. Chem. Int. Ed.* **48**:9879.
 [3] Besanceney-Webler *et al.* (2011) Increasing the Efficiency of Bioorthogonal Click Reactions for Bioconjugation: A Comparative Study. *Angew. Chem. Int. Ed.* **50**:8051.
 [4] Uttamapinant *et al.* (2012) Fast, Cell-Compatible Click Chemistry with Copper-Chelating Azides for Biomolecular Labeling. *Angew. Chem. Int. Ed.* **51**:5852.