DATA SHEET

Jena Bioscience

CuAAC Biomolecule Reaction Buffer Kit (THPTA based)

Cat. No.	Amount
CLK-072	1 kit

For general laboratory use.

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 Biomolecule Reaction Buffer Kit (THPTA based) is suitable to perform Copper (Cu(I))-catalyzed Azide-Alkyne Click chemistry reactions (CuAAC) with Azide- or Alkyne- modified biomolecules.

1 Kit provides sufficient amounts to perform **25 CuAAC experiments à 200 µl using 2 mM $CuSO_4$ (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:
1 x 10 mg $CuSO_4$ (M = 159.6 g/mol), #CLK-MI004)

Cu(I) stabilizing ligand:
1 x 25 mg THPTA (M= 434.5 g/mol, #CLK-1010)

Reduction Reagent:
1 x 200 mg Na-Ascorbate (M = 198.1 g/mol, #CLK-MI005)

Reaction Buffer:
1 x 30 ml 100 mM Na-Phosphate Buffer, pH 7

10 ml ddH_2O

Materials required but not provided:

Alkyne-or Azide-functionalized substrates e.g.
a) fixed and permeabilized cells containing metabolically functionalized Alkyne- or Azide-modified biomolecules.
b) cell lysate containing metabolically functionalized Alkyne- or Azide-modified proteins.

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

1. Introduction

Copper (**Cu**(I))-catalyzed **A**zide-**A**lkyne **C**lick 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)).

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 THPTA and sodium ascorbate as a reduction reagent is recommended.[1-3]

CuAAC Biomolecule Reaction Buffer Kit (THPTA based)

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 Biomolecule Reaction Buffer Kit (THPTA based) provides sufficient amounts to perform 25 CuAAC experiments à 200 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 biomolecules (see 3.) is outlined below. Individual optimization might however be required for different CUAAC labeling experiments as well as for critical reaction parameter e.g. final $CuSO_4$ concentration, $CuSO_4$:ligand 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

Please note: *The concentration of stock solutions (2.1 to 2.3) is suitable to prepare 200 and 500 µl assays containing 2 mM $CuSO_4$, 10 mM THPTA and 100 mM Na-Ascorbate (see 3.1 and 3.2, 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 ddH_2O (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 ddH_2O required for a 250 mM THPTA stock solution.

THPTA	Concentration of stock solution	Amount of ddH_2O
25 mg	250 mM	230 µl

2.2 $CuSO_4$ stock solution (copper source)

- Add an appropriate amount of ddH_2O (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 ddH_2O required for a 100 mM $CuSO_4$ stock solution.

$CuSO_4$	Concentration of stock solution	Amount of ddH_2O
10 mg	100 mM	628 µl

2.3 Na-Ascorbate stock solution (reduction reagent)

- Add an appropriate amount of ddH_2O (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 to slightly yellow and turn brown upon oxidization thereby losing their reduction capability.*

Table 3 Volume of ddH_2O required for a 1 M Na-Ascorbate stock solution.

Na-Ascorbate	Concentration of stock solution	Amount of ddH_2O
200 mg	1 M	1010 µl

2.4 (Picolyl)-Azide detection reagent stock solution

- (Picolyl)-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 µM for Picolyl-Azide detection reagents.
- Final concentrations of Azide or Alkyne detection reagents may

CuAAC Biomolecule Reaction Buffer Kit (THPTA based)

range from 2 µM to 100 µM. Final concentrations of Picolyl-Azide detection reagents may range from 0.5 to 5 µM (see 3.4).
- If the molar amount of Alkyne-groups can be calculated, we recommend starting with a 10-fold molar excess of Azide-functionalized detection reagent.

3. General protocol for CLICK labeling of biomolecules

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

The amount of provided reagents is sufficient to perform **25 CuAAC experiments à 200 µl** using 2 mM $CuSO_4$, 10 mM THPTA and 100 mM Na-Ascorbate in 100 mM Na-Phosphate reaction buffer.

3.1 Prepare $CuSO_4$:THPTA-Premix

Please note: *Both the final $CuSO_4$ concentration as well as $CuSO_4$:THPTA ratio are critical parameters for CuAAC reaction efficiency. A final $CuSO_4$ concentration of 2 mM and a $CuSO_4$:THPTA ratio of 1:5 is recommended as a starting point for labeling of Azide- and Alkyne-functionalized biomolecules with a correspondingly labeled detection reagent. Individual optimization for each assay is strongly recommended. Minimum $CuSO_4$ concentration: 50 µM.*

- Prepare the $CuSO_4$:THPTA-Premix freshly for each experiment.
- Allow all solutions to warm up to room temperature.
- Mix the appropriate amount of 100 mM $CuSO_4$ and 250 mM THPTA stock solution (Tab. 4) by vortexing and spin down briefly.
- 12 µl $CuSO_4$:THPTA-Premix (1 Assay) is sufficient for the preparation of 500 µl CLICK reaction assay (see. 3.2).

Table 4 Pipetting scheme for $CuSO_4$:THPTA-Premix (ratio 1:5).

Compound	Final conc.	1 Assay
100 mM $CuSO_4$ stock solution **(see 2.2)**	33.33 mM	4 µl
250 mM THPTA stock solution **(see 2.1)**	166.66 mM	8 µl

3.2 Perform CLICK labeling

Please note: *The protocol below describes CuAAC labeling of an Alkyne-functionalized biomolecule (e.g. cell lysate containing Alkyne-functionalized proteins) with an Azide-functionalized detection reagent (e.g. Azide-functionalized fluorescent dye). It can be used vice versa as well (Azide-functionalized biomolecule and Alkyne-functionalized detection reagent).*

- Allow all solutions to warm up to room temperature.
- Final assay volume: 200 µl.
- Refer to Tab. 6 for appropriate amounts of stock solutions.
- Mix an Alkyne-functionalized biomolecule (see Tab. 5) with an appropriate amount of 100 mM Na-Phosphate reaction buffer, pH 7 to achieve a final volume of 167 µl.

Table 5 Starting amount of Alkyne-functionalized biomolecules. ***Please note:*** *The stated amounts are intended for an orientation only. They may need to be adjusted depending on the final read-out or downstream processing after CLICK reaction.*

Substrate	Final Amount	Recommended final assay volume
Cell lysate containing Alkyne-functionalized proteins	50 µg	200 µl
Single Alkyne-functionalized oligonucleotide	5 - 10 nmol	20-50 µl
Multiple Alkyne-functionalized DNA or RNA fragments generated by enzymatic incorporation of correspondingly labeled nucleotides	3 - 15 pmol*	20-50 µl

*e.g. 3 pmol correspond to 1.5 µg of a 1500 bp RNA fragment or 1 µg of a 500 bp DNA fragment.

- Add 1 µl of a 10 mM Azide- functionalized detection reagent stock solution (see 2.4) vortex and spin-down briefly (final concentration: 50 µM). If the molar amount of Alkyne-groups can be calculated, we recommend starting with a 10-fold molar excess of Azide-functionalized detection reagent.
- Add 12 µl $CuSO_4$/THPTA-Premix (see. 3.1), vortex and spin down briefly.
- Add 20 µl 1 M Na-Ascorbate stock solution (see 2.3) to initiate the reaction, vortex and spin down briefly.
- Incubate samples 30 – 60 min at room temperature or 37 °C (protected from light).
- Alkyne-functionalized biomolecules are now CLICK-labeled and ready for downstream processing and/or analysis.

CuAAC Biomolecule Reaction Buffer Kit (THPTA based)

Table 6 Pipetting scheme for a 200 µl CLICK reaction assay. Please add the compounds exactly in the order described below.

Compound	Final conc./amount	1 Assay (200 µl)
Alkyne-functionalized biomolecule	see tab. 5	X µl
100 mM Na-Phosphate reaction buffer, pH 7	100 mM	ad 167 µl
10 mM Azide-functionalized detection reagent stock solution **(not provided, see 2.4)**	50 µM[1]	1 µl
33.33 mM / 166.66 mM $CuSO_4$:THPTA-Premix **(see 3.1)**	2 mM / 10 mM	12 µl
1 M Na-Ascorbate stock solution **(see 2.3)**	100 mM	20 µl

[1]Final concentrations of Azide or Alkyne detection reagents may range from 2 µM to 100 µM. Final concentrations of Picolyl-Azide detection reagents may range from 0.5 to 5 µM. We recommend starting with 50 µM or 5 µM, respectively in case the molar amount of Alkyne-groups is unknown. If the molar amount of Alkyne-groups can be calculated, we recommend starting with a 10-fold molar excess of Azide-functionalized detection reagent. Concentrations can be titrated down in case of high background or up in case of low signal.

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 Efficiacy 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.