# CuAAC Biomolecule Reaction Buffer Kit (THPTA based)

# Cat#: orb532322 (Product Manual)

## 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)).

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]

The use of Picolyl-Azide reagents instead of conventional Azide reagents can further increase the reaction efficiency and decrease the required final CuSO4 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:

1) Prepare a mix of Alkyne- and Azide functionalized molecules in an appropriate reaction buffer.

2) Prepare a CuSO4: Cu(I)-ligand premix, add it to the Azide- Alkyne solution and mix briefly.

3) 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 CuSO4, 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 CuSO4 concentration, CuSO4:ligand ratio, detection reagent concentration.

Hong et al.[2] and Presolski et al.[1] provide useful background information on the influence of CuSO4 concentration, CuSO4: 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 CuSO4, 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 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 $ddH_2O$ |
|---|---|---|
| 25 mg | 250 mM | 230 µl |

### 2.2 CuSO4 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 CuSO4 stock solution.

| $CuSO_4$ | Concentration of stock solution | $ddH_2O$ to be added |
|---|---|---|
| 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 | $ddH_2O$ to be added |
|---|---|---|
| 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 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 CuSO4, 10 mM THPTA and 100 mM Na -Ascorbate in 100 mM Na-Phosphate reaction buffer.

### 3.1 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 CuSO 4 concentration of 2 mM and a CuSO4: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 CuSO4 concentration: 50 µM.

- 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.
- 12 µl CuSO4:THPTA-Premix (1 Assay) is sufficient for the preparation of 500 µl CLICK reaction assay (see. 3.2).

**Table 4 Pipetting scheme for CuSO4: 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 | Recommend ed 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 CuSO4/THPTA-Premix (see. 3.1), vortex and spin down briefly.

- Add 20 µl 1M 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.

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.