CuAAC Cell Reaction Buffer Kit (BTTAA based)

Cat #: orb532323 (manual)

For research use only. Not intended for diagnostic use.

Background

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 CuSO4 as copper source, a water-soluble Cu(I) stabilizing ligand such as BTTAA and sodium ascorbate as a reduction reagent is recommended. 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.

Kit Components

Reagents	Cat #	Quantity
Copper source: CuSO4 (M = 159.6 g/mol)	orb532416	2 x 10 mg
Cu(I) stabilizing ligand: BTTAA (M= 430.5 g/mol)	orb1734963	5 x 25 mg
Reduction Reagent: Na-Ascorbate (M = 198.1 g/mol)	orb532417	4 x 200 mg
Reaction Buffer: 100 mM Na-Phosphate Buffer, pH 7		2 x 30 ml
ddH2O		10 ml

Materials Required but Not Supplied

1. Alkyne-or Azide-functionalized substrates e.g. fixed and permeabilized cells containing metabolically functionalized Alkyne- or Azide-modified biomolecules.
2. (Picolyl)-Azide or Alkyne detection reagent and appropriate solvent (e.g. DMSO)
3. 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

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Assay Procedure

1.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 CuSO4: 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 (BTTAA based) provides sufficient amounts to perform 50 CuAAC experiments à 500 µl using 2 mM CuSO4, 10 mM BTTAA 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. However, individual optimization might be required for different CUAAC labeling experiments as well as for critical reaction parameter e.g. final CuSO4 concentration, CuSO4:BTTAA ratio, detection reagent concentration.

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 CuSO4, 10 mM BTTAA 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 BTTAA 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.
- BTTAA is less soluble than THPTA. If required, heat shortly up to 70 °C to achieve a clear solution.

Table 1 Volume of ddH2O required for a 50 mM BTTAA stock solution.

BTTAA	Concentration of stock solution	Amount of ddH2O
25 mg	50 mM	1163 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.

CuSO4	Concentration of stock solution	Amount of ddH2O
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 to slightly yellow 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 ddH2O
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).

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:BTTAA-Premix

Please note: Both the final CuSO4 concentration as well as CuSO4:BTTAA ratio are critical parameters for CuAAC reaction efficiency. A final CuSO4 concentration of 2 mM and a CuSO4:BTTAA 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 µM.

- Prepare the CuSO4:BTTAA-Premix freshly for each experiment.
- Allow all solutions to warm up to room temperature.
- Mix the appropriate amount of 100 mM CuSO4 and 50 mM BTTAA stock solution (Tab. 4) by vortexing and spin down briefly.
- 110 µl CuSO4:BTTAA-Premix (1 Assay) is sufficient for the preparation of 500 µl CLICK reaction cocktail (see. 3.4).

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

Compound	Final conc.	1 Assay	10 Assays
100 mM CuSO4 stock solution (see 2.2)	9.1 mM	10 µl	100 µl
50 mM BTTAA stock solution (see 2.1)	45.45 mM	100 µl	1000 µl

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 µl 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 µl 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/BTTAA Premix, vortex and spin down briefly.
- Add Na-Ascorbate, vortex and spin down briefly.

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

Compound	Final conc.	1 Assay (500 µl)	10 Assays (5 ml)
100 mM Na-Phosphate reaction buffer, pH 7		339 µl	3.39 ml
10 mM Azide or-Alkyne detection reagent stock solution (not provided, see 2.4)	20 µM	1 µl	10 µl
9.1 mM / 45.45 mM CuSO4:BTTAA-Premix (see 3.3)	2 mM / 10 mM	110 µl	1100 µl
1 M Na-Ascorbate stock solution (see 2.3)	100 mM	50 µl	500 µl

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 20 µM or 5 µM, 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 µl 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.

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