# BaseClick™-Kit Eterneon 394/507

(BCK-009)

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**Literature Citation:** When describing a procedure for publication using this product, please refer to it as the BaseClick™-Kit.

We recommend using the following general protocol for click chemistry labeling of alkyne-modified oligonucleotides with azides produced by baseclick GmbH. The auxiliary reagents can be ordered at baseclick GmbH separately.

*Protocol*

## A. General Considerations

- This protocol is optimized for the labeling of 5 nmol of a single alkyne-modified oligonucleotide via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc; Click Chemistry). Nevertheless, it can be scaled up easily.

- The BaseClick™-Kit Eterneon 394/507 contains chemicals to perform six different sets of click reactions. The reagents in this kit can be used to label at least 370 nmol of terminal alkyne-functions with Eterneon™ 394/507 Azide.

- The labeling reaction works more efficiently with concentrated solutions of alkynes (oligo) and azides (label).

- The best way to carry out the click reaction is to mix the oligo and the azide-label in a minimal amount of solvent.

- Alkyne / azide ratio: from 1:2 to 1:5. For highdensity labeling reactions (e.g. > 4 alkynes in a row) an alkyne / azide ratio of 1:10 is recommended.

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- The click reaction is normally accelerated by elevated temperature and can be finished in less than 30 min when the reaction temperature is around 40 - 45 °C. The reaction works perfectly at room temperature as well and can even be lowered to 1 °C if required.

- The reaction time depends on: a) concentration of azide and oligo in the solution; b) reaction temperature; c) stirring and/or mixing of the solution.

- Excess of reagents are normally removed by ethanol precipitation of the labeled oligonucleotide.

## B. Materials provided with the Kit and storage conditions

| Vial-colour | Amount | Component | Storage |
|---|---|---|---|
| red | 1 mg | Eterneon™ 394/507 Azide[2] | dark, -20 °C |
| yellow | 5,4 mg | TBTA-Ligand[1, 2] | -20 °C |
| green | 6 x 1 mg | CuBr[1] | dry, inert gas |
| blue | 2 x1 mL | DMSO/t-BuOH[1] | RT |

1 baseclick grade
2 a prepared solution can be stored at - 20 °C in the dark for several months
(Note: The azide functionality is very stable and does not hydrolyze in the presence of water.)

## C. Required Material and Equipment

Alkyne-modified oligonucleotide (can be ordered @ baseclick GmbH, Integrated DNA Technologies, Metabion, IBA or EllaBiotech)
Microcentrifuge
Vortexer
Thermomixer (optional)
Microcentrifuge tubes
Ethanol (5 % Diethylether)
0,3 M Sodium-acetate solution (0,3 M NaOAc)

# D. Click-Protocol for Oligonucleotide Labeling

## 1. Before You Start

a) Calculate the total amount of terminal alkyne modifications *n* in nmol present in your oligonucleotide:

$$n = m \times y$$ where:

*n* = terminal alkyne modifications in nmol
*m* = amount of oligonucleotides in nmol
*y* = number of terminal alkyne functions in your oligonucleotide

e.g. you have 20 nmol of a triple alkyne modified oligonucleotide:

amount of terminal alkyne modifications *n* = 20 nmol **x** 3 = 60 nmol.

b) Calculate the amount of azide *k* in nmol required for the labeling of the alkyne-modified oligonucleotide:

$$k = n \times l$$ where:

*k* = amount of azide required for labeling in nmol
*n* = terminal alkyne modifications in nmol
*l* = azide equivalents (excess of azide: between 2 and 10)

e.g. you have *n* = 60 nmol of terminal alkyne modifications:

amount of azide *k* = 60 nmol **x** 5 = 300 nmol.

## 2. Preparation of the *Oligonucleotide Solution*

Dissolve the oligonucleotide in the appropriate amount of water to adjust to 1 mM solution and centrifuge shortly. (Also different concentrations can be used!).

## 3. Preparation of a 10 mM *Eterneon™ 394/507 Azide Solution*

a) Take the **red vial** with 1 mg Eterneon™ 394/507 Azide out of the freezer and slowly warm up to room temperature.

b) Centrifuge shortly to place all Eterneon™ 394/507 Azide on the bottom of the vial.

c) Pipette 185 µL of DMSO/*t*-BuOH-solution (**blue vial**) into the **red vial** with Eterneon™ 394/507 Azide.

d) Vortex the **red vial** until the azide is dissolved completely.

e) Centrifuge shortly. This solution can be stored at -20°C in the dark for several months. The azide functionality is very stable and does not hydrolyze in the presence of water.

## 4. Preparation of *Oligonucleotide / Eterneon™ 394/507 Azide Solution*

a) Prepare a 1,5 mL vial (referred to as **reaction vial**).

b) Pipette the correct amount of oligonucleotide solution corresponding to 5 nmol of oligonucleotide into the **reaction vial** (here: 5 µL of a 1 mM solution).

c) Pipette 2,5 µL of Eterneon™ 394/507 Azide solution (**red vial**, 10 mM, 25 nmol, 5 equivalents) directly into the **reaction vial** with the oligonucleotide.

## 5. Preparation of the *TBTA-Ligand Solution*

a) Dissolve the whole content of the **yellow vial** (TBTA-Ligand) in 100 µL DMSO/*t*-BuOH (**blue vial**). This solution can be stored at -20°C for several months.

b) If this solution was already prepared, take it out of the freezer and let it defrost. Vortex shortly and centrifuge.

c) Pipette 10 µL out of the **yellow vial** into an empty vial (referred to as **catalyst vial**).

***Please notice that the next three steps (6, 7 and 8) have to be performed as quick as possible!!***

## 6. Preparation of the *CuBr Solution*

*This solution must always be freshly prepared prior to use!*

a) Do not open the **green vial** before using it! CuBr is stored under protective atmosphere (inert gas, Argon). Centrifuge the **green vial** shortly to place all CuBr on the bottom of the vial.

b) Pipette 70 µL DMSO/*t*-BuOH-solution (**blue vial**) into the **green vial** and dissolve the CuBr completely by vortexing. Centrifuge shortly. **Be quick to avoid oxidation of Cu(I)! Proceed immediately with the next step!**

**7. Preparation of the *Catalyst Solution***

*This solution must always be freshly prepared prior to use!*

Pipette 5 µL of the freshly prepared CuBr solution (**green vial**, see step 6 b)) into the **catalyst vial** with the TBTA-Ligand (see step 5 c)) and vortex for 10 sec. Centrifuge shortly. **Be quick to avoid oxidation of Cu(I)! Proceed immediately with the next step!**

**8. Performing the *Click Reaction***

Pipette 1,5 µL of the *Catalyst Solution* (**catalyst vial**, see step 7) into the **reaction vial** (*Oligonucleotide / Eterneon™ 394/507 Azide Solution*, see step 4) and vortex for 10 sec. Centrifuge shortly and place the **reaction vial** in a Thermomixer at 37 °C. Shake the **reaction vial** for 4 h. (Alternatively the **reaction vial** can be placed in a warm water bath.)

**9. Removal of excess reagents**

Add 100 µL of a 0,3 M NaOAc solution and precipitate the oligonucleotide by adding 1 mL cold ethanol (containing 5 % diethylether; -20 °C). Centrifuge for at least 20 min at 6000 rpm or higher. Remove the supernatant and wash the residue with 100 µL cold ethanol (containing 5 % diethylether; -20 °C). Centrifuge again for at least 10 min and remove the supernatant. Dry the residue on air.

## E. Troubleshooting

If significant precipitation of azide or ligand is observed, add additional DMSO/*t*-BuOH-solution and/or heat up gently until all components are dissolved completely.

If the *Click Reaction* was not successful, check your CuBr. If the freshly prepared CuBr-solution has already a deep brown colour, the CuBr is significantly oxidized. Repeat the reaction using a new batch of CuBr.

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