Oligo-Click-M Reload

(BCK-OligoM-R)

For Click Chemistry labeling of up to 100 nmol oligonucleotide containing 1 to 2 alkynes.
9 Reactions

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Literature citation:
When describing a procedure for publication using this product, please refer to it as the Baseclick Oligo-Click.

We recommend to use the following general protocol for click chemistry labeling of alkyne-modified oligonucleotides (from 10 to 100 nmol) with Label-Azides provided by baseclick GmbH. The Label-Azides and the other auxiliary reagents can be ordered at baseclick GmbH separately.

Protocol

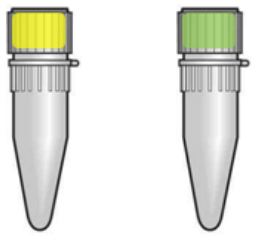
A. General considerations

- This protocol is optimized for the labeling of up to **100 nmol** of a single or double alkyne-modified oligonucleotide via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAc; Click Chemistry).
- The Reactor-M vial contains a stable **heterogeneous catalyst**, which won't be dissolved during the reaction.
- The labeling reaction works more efficiently with concentrated solutions of alkynes (oligo) and azides (Label-Azide, L-N_3).
- The best way to carry out the click reaction is to mix the oligo and the Label-Azide in a minimal amount of solvent.
- The click reaction is normally accelerated by elevated temperatures and can be finished in 30 min when the reaction temperature is 45°C. Low reaction temperatures (e.g. 4 °C) can be applied as well in combination with longer reaction time.
- 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: d) azide steric demand for double-labeling reactions. In the latter case use a prolonged (4 h) reaction time.

B. Materials and storage conditions for up to nine (9) independent labeling reactions provided with the Oligo-Click-M Reload.

Vial colour	Quantity	Name	Amount	Storage
yellow	1	Activator *	50 µL	-20°C
green	9	Reactor-M	N.A.	RT

* Contains DMSO. Download the MSDS from the baseclick website.



1 x 9 x

C. Required Material and Equipment – not provided with this Kit

Alkyne-modified oligonucleotide or Alkyne-modified PCR fragment
Label-Azide (10 mM)
Centrifuge (optional refrigerated)
Microcentrifuge tubes
Thermomixer (optional)
Ethanol 95%
3M Sodium-acetate solution (3M NaOAc) or ammonium-acetate 3M NH_4OAc.

D. Click protocol for Oligonucleotide and PCR labeling

1. Preparation of the Oligonucleotide or PCR fragment solution (not provided with the KIT)

Dissolve the oligonucleotide in the appropriate amount of water to adjust to a 0.1 - 1 mM solution and centrifuge shortly. (Also different concentrations can be used, see Reaction Table at page 5).

or

Dissolve the PCR fragment in an appropriate amount of water or buffer (**avoid** EDTA and EDTA-containing buffers) to adjust to ca. 50 – 150 ng/µL solution. For more information refer to the baseclick PCR-Click Kits and the corresponding user manuals available under www.baseclick.eu.

2. Preparation of a 10 mM Label-Azide (L-N_3) solution[1]

(Select your preferred Oligo-Click / Azide combination from the baseclick website)

2.1 Take 1 mg of your selected azide L-N_3 out of the freezer and slowly warm up to room temperature.

2.2 Centrifuge shortly to place all L-N_3 on the bottom of the vial.

2.3 Pipette (100,000 / $Mw_{L\text{-}N3}$) µL of the click solvent[2] into the vial containing the Label-Azide.[3]

2.4 Vortex the vial until the Label-Azide is dissolved completely.

2.5 Centrifuge shortly.

[1] This preparation is valid for Label-Azides (not included in this kit) soluble in DMSO. You can also use pure water or other solvents compatible with the Label-Azide you selected (see baseclick azides under www.baseclick.eu)

[2] This solvent contains a DMSO/*t*-BuOH mixture. Download the MSDS from the baseclick website (Product Code BCMI-003).

[3] The molecular weight $Mw_{L\text{-}N3}$ is reported on the red vial and in the corresponding Label-Azide Data-Sheet. See also the calculation sheet on page 7.

3. Performing the click reaction (1-2 min. preparation + 1 h reaction)
(Be aware that the catalyst is solid and will not be dissolved during the click reaction!)

[Step 1] Pipette **5 µL** of the activator (**yellow vial**) into the **green vial**

[Step 2] Pipette the appropriate amount of the oligo or DNA solution[4] into the **green vial** from Step 1

[Step 3] Pipette the correct amount[5] of Label-Azide solution reported in the Reaction Table at page 5 into the **green vial** from Step 2

[Step 4] Gently vortex the **green vial** from Step 3 for 10 sec. Centrifuge shortly

[Step 5] Place the **green vial** from Step 4 in a thermomixer at 45°C for 1h under gentle shaking (do not exceed 700 rpi) or in a water bath at 45 °C for 1 h. You can run the reaction at room temperature (RT) as well. In this case use a prolonged reaction time (2-4 h).

IMPORTANT: Provide always some mixing over the reaction time.

4. Work up (15 – 20 min.)

[Step 7]

4.1 Transfer only the liquid phase into a **new empty vial**

4.2 Wash the **green vial** containing the solid catalyst with 60 µL of 3M NaOAc

4.3 Collect only the liquid phase from point 4.2 in the **new empty vial** containing your labeled-oligonucleotide from step 4.1

Proceed with your preferred DNA precipitation or continue with point 5:

5. Precipitation protocol

[Step 8]

5.1 Add 1 mL cold ethanol 95%

5.2 Centrifuge for at least 15 min at 4°C or cool for 1 h at -20 °C and then centrifuge

5.3 Remove the supernatant and dry the residue on air

5.4 Re-dissolve the pellets in the desired amount of water or buffer

Your labeled-oligonucleotide or DNA is ready for your experiment / assay. The final product may contain traces of free Label-Azide, although most of the reagents have been removed during the precipitation step.. Applicable purification methods: 1. Desalting. 2. RP-HPLC. 3. Gel Electrophoresis.

[4] See "Minimal Oligo Conc." and " Maximal Reaction Volume" in Reaction Table on page 5.
[5] See Reaction Table at page 5 or the calculation sheet on pages 8-9.

Reaction Table:

Use the following table to calculate the amount of reagents (Activator and Azide) you need in your oligonucleotide labeling click reactions you in a fast and very reliable way.[6]

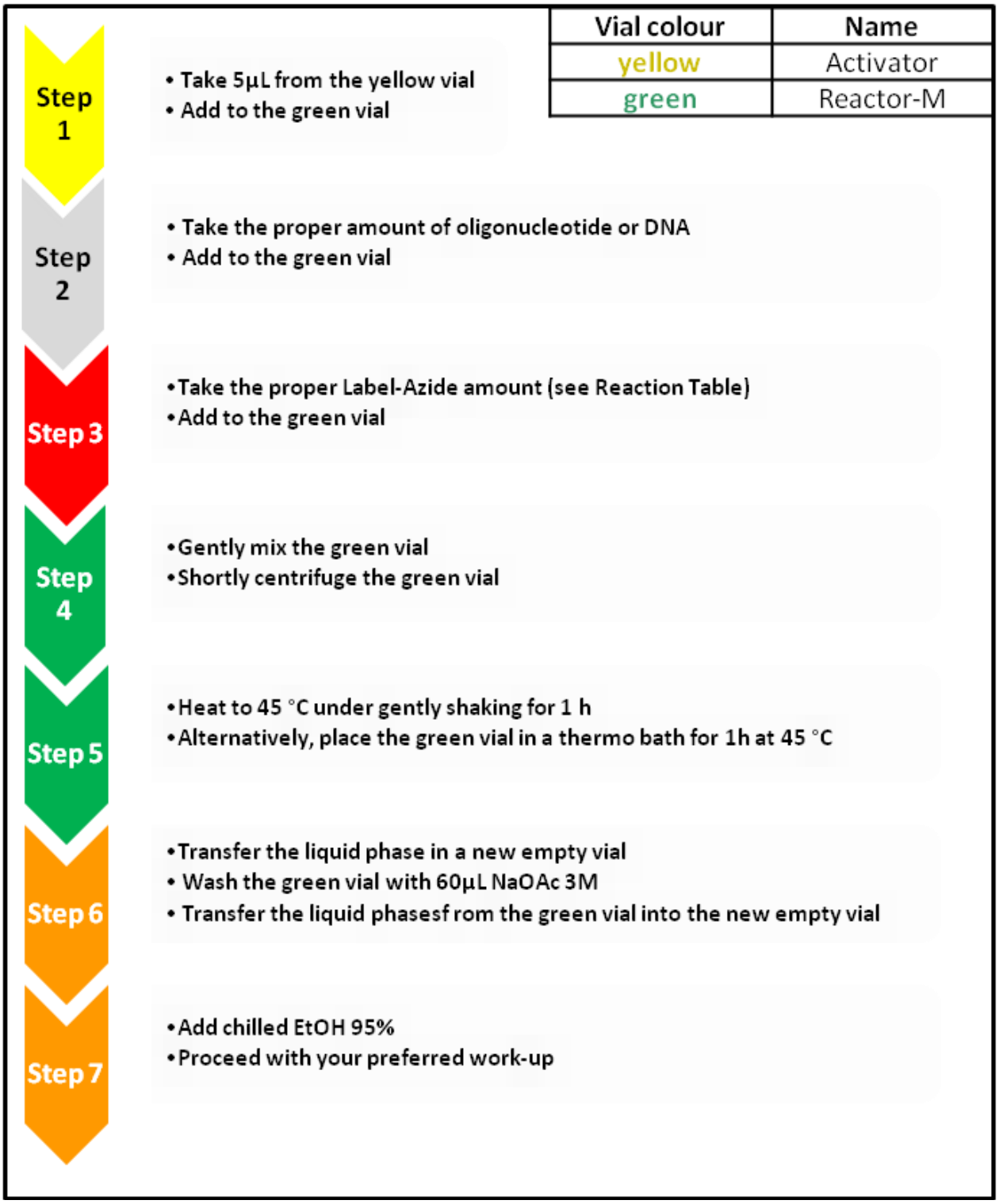
You will need different amounts of Label-Azide – "*Azide µL (Red)*" column - depending on the amount of oligonucleotide – "*Oligo nmol range*" column - and the amount of alkynes present in your sequence – "*Alkyne content range*" column.

Add the reagents as described in Point 3 of this protocol.

Oligo nmol range	Alkyne content range	Activator µL (Yellow)	Azide µL (Red)	Reactor (Green)	Minimal Oligo Conc.	Maximal reaction volume in µL
11 - 30	*For a 22mer this range corresponds to **2.5 – 6.6 OD** or **73 – 200 µg***					
	1 - 2	**5**	**12**	M	0.1 mM	150
31 - 50	*For a 22mer this range corresponds to **7.0 - 11 OD** or **205 - 330 µg***					
	1 - 2	**5**	**20**	M	0.1 mM	300
51 - 70	*For a 22mer this range corresponds to **11 - 16 OD** or **337 - 462 µg***					
	1 - 2	**5**	**28**	M	0.1 mM	300
71-100	*For a 22mer this range corresponds to **16 - 22 OD** or **337 - 470 µg***					
	1 - 2	**5**	**40**	M	0.1 mM	300

[6] For a detailed calculation see page 8 of this user manual. Use the Azide Table on pages 10 in order to minimize the amount of Label-Azide required in your labeling reaction.

Work Flow



Vial colour	Name
yellow	Activator
green	Reactor-M

Appendix

E Calculation Sheet

1 Preparation of a 10 mM Label-Azide (L-N_3) Solution

To calculate the amount of solvent V_L in µL to be added to 1 mg of Label-Azide (L-N_3) to prepare a 10mM solution divide 100,000 by the molecular weight of the Label-Azide ($Mw_{L\text{-}N3}$).

E.g.:

- m = Label-Azide = FAM-N_3 1 mg
- $Mw_{L\text{-}N3}$ = 458.4 g/mol
- V_L = 100,000 / 458.4 = 218.2 µL
- c_{azide} = 10 mM

1.1 Take 1 mg Label-Azide out of the freezer and slowly warm up to room temperature.

1.2 Centrifuge shortly to place all the Label-Azide on the bottom of the vial.

1.3 Pipette V_L (µL calculated in 1) of click solvent into the vial with the Label-Azide.

1.4 Vortex the vial until the Label-Azide is dissolved completely.

1.5 Centrifuge shortly. This solution can be stored at -20°C in the dark for several months (refer to the Label-Azide Data-Sheet). The azide functionality is very stable and does not hydrolyze in water.

F Click reaction calculation sheet

Use the **Reaction Table** on page 5 to read out the amount of Label-Azide (L-N_3) to be used in your experiment. Use the **Azide Table** on page 10 if you need to minimize the amount of Label-Azide used in your labeling reaction. Below you can read how you can calculate those values yourself:

1. *For oligonucleotide labeling*:

1.1 Calculate the amount of oligonucleotide n_{oligo} in nmol

- n_{oligo} [nmol] = m [ng] / Mw [g/mol]
- n [nmol] = c [mM] x V [µL]

1.2 If you have a concentrations c [ng/µL] divide this value by the molecular weight Mw [g/mol] of your oligo in order to obtain the total concentration in nmol/µl. Multiply this value by the total volume in µl to obtain the total amount of your oligo n_{oligo} in nmol.

Example:
oligonucleotide containing two (2) alkynes and the following specifications:

- c_{oligo} = 250 ng/µL
- Mw_{oligo} = 6500 g/mol
- Total volume = V_{oligo} = 150 µL
- Total amount = n_{oligo} = (250 / 6500) x 150 = 5.8 nmol

1.3 Multiply n_{oligo} by the total amount of incorporated alkynes in order to obtain $n_{alkynes}$ in nmol.

- Oligo containing 2 alkynes
- n_{oligo} = 5.8 nmol
- $n_{alkynes}$ = 5.8 x 2 = 11.6 nmol

1.4 The click reaction requires only two equivalents of azide. Multiply $n_{alkynes}$ x 2 to obtain n_{azide} in nmol.

- n_{azide} = 11.6 x 2 = 23.2 nmol

1.5 Divide n_{azide} by the azide concentration c_{azide} = 10 mM in order to obtain the amount of azide (V_{azide} in µL) to be used in the reaction.

- V_{azide} = n_{azide} / c_{azide} = 23.2 / 10 = 2.3 µL
- Use 2.3 µL of Label-Azide 10 mM in your click reaction.

2. *For PCR labeling:*

(refer also to the baseclick PCR-Click Kit user manual under www.baseclick.eu)

Calculate the amount of Azide (L-N_3) that you want to use for labeling your alkyne-modified DNA. The final labeling rate of the DNA can be tuned by the amount of azide used and has to be adjusted for every new DNA template.

2.1 Measure the DNA concentration c_{DNA} [ng/µL] after PCR workup with a photometer.

2.2 Calculate the molecular weight Mw (g/mol) of your DNA template (Mw_{DNA}):

Mw_{DNA} [g/mol] = 600 g/mol x bp

- 600 g/mol is the average mass of a basepair
- bp = number of basepairs in your DNA template

2.3 Calculate the total amount of DNA n_{DNA} in nmol present in your sample:

n_{DNA} [nmol] = c_{DNA} [ng/µL] x V_{DNA} [µL] / Mw [g/mol]

- c_{DNA} [ng/µL]: measured in 2.1
- Mw_{DNA} [g/mol]: calculated in 2.2
- V_{DNA} [µL] = volume of your sample (measure it with a pipette)

2.4 Calculate the total amount of terminal alkyne modifications $n_{alkynes}$ in nmol in your DNA. This amount corresponds to the amount of Thymidines in your DNA if dTTP was replaced by **C8-Alkyne-dUTP** during PCR:

$n_{alkynes}$ [nmol] = [(bp x AT-content %) / 100] x n_{DNA} [nmol]

- bp = number of basepairs in your DNA template
- AT-content % = percentage of A's and T's in your DNA
- n_{DNA} (nmol) = calculated in 2.3

If dCTP was replaced by **C8-Alkyne-dCTP** during PCR then calculate $n_{alkynes}$ in nmol in your DNA as follow:

$n_{alkynes}$ [nmol] = (bp x GC-content %) / 100 x n_{DNA} [nmol]

- bp = number of basepairs in your DNA template
- GC-content % = percentage of G's and C's in your DNA
- n_{DNA} [nmol] = calculated in 2.3

2.5 Calculate the amount of Label-Azide n_{azide} in nmol for labeling the alkyne-modified DNA. Labeling rates depend on the amount of Label-Azide applied. Normally 1 – 30 equivalents of azide are used, resulting in labeling rates of up to 20 % and more!

n_{azide} [nmol] = $n_{alkynes}$ [nmol] x k

- $n_{alkynes}$ [nmol] = calculated in 2.4
- k = equivalents of azide (normally 1 – 30)

V_{azide} (Label-Azide; 10 mM) = n_{azide} [nmol] / 10 nmol/µL

Add V_{azide} [µL] to your click reaction.

Appendix

Azide Table

Use these tables to read out the **minimum amount** of Label-Azide needed in your labeling click reaction, in order to reduce the Label-Azide consumption when needed.

For example, if you have 65 nmol of an oligonucleotide (nmol Oligo = 65) containing 2 alkynes in the sequence (Nr. of Alkynes = 2) then use 26 µL of the Label-Azide 10 mM solution.[7]

Nr. of Alkynes		1	2
nmol Oligo		µL Azide	µL Azide
15		3	6
20		4	8
25		5	10
30		6	12
35		7	14
40		8	16
45		9	18
50		10	20
55		11	22
60		12	24
65		13	26
70		14	28
75		15	30
80		16	32
85		17	34
90		18	36
95		19	38
100		20	40

Troubleshooting

If the labeling is not complete then increase the reaction time and eventually the reaction temperature (recommended for multi labeling reactions and/or for azides with high steric demand).

[7] The amount of Label-Azide reported in the Reaction Table at page 5 are for this example 28 µL, which cover the range between 51 and 70 nmol oligo containing from 1 to 2 alkynes in the sequence.