User Manual

ClickTech PCR Modification Kit

Version 1.0

Ordering information

(for detailed kit content see **Table 1**)

ClickTech PCR Modification Kits:

Product Number	Used fluorescent dye	Excitation (nm)	Emission (nm)	Spectral range
BCK-PCR-MOD-488	6-FAM Azide (FITC alternative)	496	516	**green**
BCK-PCR-MOD-555	5-TAMRA-PEG3-Azide (Cy3 alternative)	546	579	**yellow**
BCK-PCR-MOD-594	5/6-Sulforhodamine 101-PEG3-Azide (Texas Red alternative)	584	603	**orange**
BCK-PCR-MOD-647	Eterneon-Red 645 Azide (Cy5 Azide alternative)	643	662	**red**

For References, FAQs and ordering please see online or contact us:

online: www.baseclick.eu

orders: orders@baseclick.eu

support: support@baseclick.eu

phone: +49 89 9699 3401

fax: +49 89 9699 4696

ClickTech PCR Modification Kit

The ClickTech PCR Modification Kits contain chemicals to perform 20 PCR reactions (50 µL each) and 40 independent labeling reactions.

Introduction and product description:

baseclick's ClickTech PCR Modification Kit contains all reagents required for generating highly labelled PCR products *via* an easy-to-handle two-step method based on click chemistry.

The Mixture of dNTP and EdUTP can be used in PCR reactions with the optimized *baseclick Ethynyl Polymerase*. The resulting PCR products contain alkyne moieties, which react with azido-derivatives of fluorescent dyes, haptnes and other labels in a highly selective fashion under benign click reaction conditions.

For research use only.

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Please read the material safety data sheets (MSDS) provided for each product/component.

Literature Citation: When describing a procedure for publication using this product, please refer to it as baseclick *ClickTech PCR Modification Kit*.

1. Materials Provided with the Kit and Storage Conditions

Table 1: Contents of the kits and storage conditions

Color code	Amount	Component	Storage
blue	25 µL	dNTP Mix (10 mM)	– 20 °C
white	25 µL	EdUTP (5 mM)	– 20 °C
purple	35 µL	baseclick Ethynyl Polymerase (2 U/µl)	– 20 °C
orange	300 µL	PCR Buffer (5x)	– 20 °C
yellow	150 µL	10x Activator[2]	– 20 °C
red	50 µL	• 6-FAM Azide[(1)] • 5-TAMRA-PEG3-Azide[(1)] • 5/6-Sulforhodamine101-PEG3-Azide[(1)] • Eterneon Red Azide[(1)]	– 20 °C, dark
green	41x	Reactor 25	RT, dry (do NOT freeze)

[(1)] The solution can be stored at – 20 °C in the dark for several months. (Note: The azide functionality is very stable and does not hydrolyse in the presence of water.)

2. Required Material and Equipment <u>not included</u> in this Kit

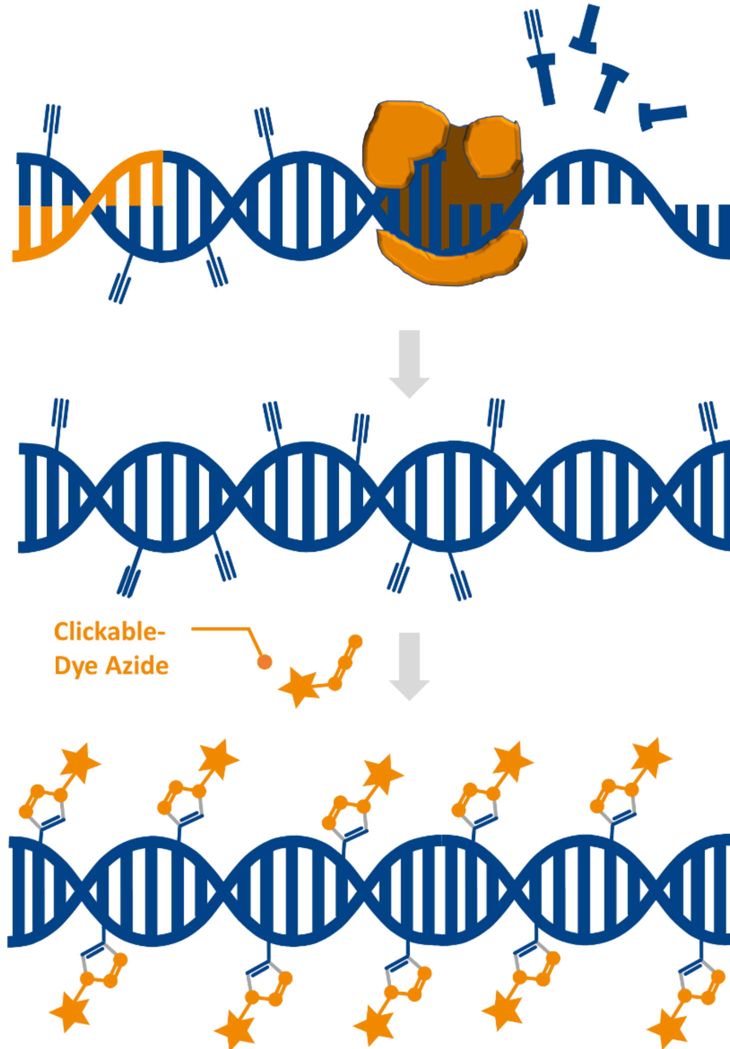
- Primers (specific primers can be ordered as a custom synthesis at baseclick, more information at www.baseclick.eu) and Template
- Reaction tubes (size depends on the volume of reaction cocktail needed)
- DNA purification kit / columns (e.g. PCR purification kit from Qiagen)
- PCR grade water
- Thermocycler
- Microcentrifuge
- Thermomixer or water bath

3. Workflow

1. Enzymatic synthesis -PCR
Native (dNTPs) and alkyne-modified (EdUTP) nucleoside triphosphates are used by our special baseclick-Ethynyl Polymerase….

…to produce a clickable PCR product.

2. Click reaction
By using the click reagents and a clickable dye of your choice you obtain DNA fluorescent labeled PCR product



4. General Considerations

- This manual contains protocols for the synthesis and labelling of alkyne-modified DNA *via* polymerase chain reaction (PCR) and subsequent click reaction.
- Excess of reagents are normally removed by spin column purification of the labelled DNA. We recommend using a PCR purification kit (e.g. QIAquick PCR Purification Kit, Qiagen) for the workup of the click reaction. Please consider fragment cut-off size when using spin column purifications.

5. PCR conditions

The optimal conditions for PCR vary depending on DNA template and primers. Especially cycle times and temperatures have to be optimized for every primer/template pair.

20 PCR reactions (50 µL each) can be performed with the material provided in this kit.

The standard setup for PCR is given below:

Table 2: Setup for a 50 µL standard PCR reaction:

Color code	Reagent	Amount	Final concentration
not included	PCR grade water	Up to 50 µL final volume	n.a.
orange	5x PCR Buffer	10 µL	1x
not included	Forward Primer (10 µM)	2.5 µL	0.5 µM
not included	Backward Primer (10 µM)	2.5 µL	0.5 µM
not included	Template DNA	1 pg - 10 ng	n.a.
white	EdUTP	1 µL	0.1 mM
blue	dNTP Mix	1 µL	0.2 mM (each dNTP)
purple	baseclick Ethynyl Polymerase	1.5 µL (3 Units)	n.a.

6. PCR-Program / Cycling

Cycle Step	Temperature	Time	Cycles
Initial denaturation	98 °C	30 s	1
Denaturation	98 °C	5 - 10 s	25 - 35
Annealing	X °C	10 - 30 s	
Extension	72 °C	15 - 30 s/kb	
Final extension	72 °C	5 - 10 min	1
Hold	4 °C	Hold	Hold

7. Click Protocol for DNA Labelling

Table 3: Setup for a standard click reaction.

Color code	Component	Amount
green	Reactor 25 (Solid Catalyst)	n.a.
yellow	10x Activator[2]	2.5 µL
not included	DNA solution (100 ng/µL)	20 µL
red	Dye azide (10 mM in DMSO)	1 µL
not included	PCR grade H_2O	1.5 µL

- Centrifuge the **green vial** with the solid catalyst shortly to place all catalyst on the bottom of the vial.
- Pipette 2.5 µL of the 10x Activator[2] (**yellow vial**) into one of the **green vials** with the catalyst. (Be aware that the catalyst is solid and will not be dissolved during the click reaction!)
- Pipette 20 µL of the DNA-solution in the same **green vial** with the solid catalyst and the Activator[2].
- Add 1 µL of the dye azide solution (**red vial**, 10 mM in DMSO) into the **green vial** with the solid catalyst, the Activator[2] and the DNA.
- Vortex shortly, spin down and place the vial in a thermomixer. Incubate the reaction for 30 min at 45 °C and 600 rpm. You can also use a water bath, but ensure some mixing within the 30 min.
- Take the supernatant for further purification. We recommend to use a PCR purification kit (e.g. QIAquick PCR Purification Kit, Qiagen) for workup of the click reaction. It is important to remove the remaining Dye-Azide after the click reaction.

Table 4: Standard dye azides contained in the different kit variants.

Product number	Dye	Excitation/ Absorption (nm)	Emission (nm)
BCFA-001	6-FAM Azide	496	516
BCFA-037	5-TAMRA-PEG3-Azide	546	579
BCFA-044	5/6-Sulforhodamine 101-PEG3-Azide	584	603
BCFA-201	Eterneon-Red 645 Azide (Cy5 Azide alternative)	643	662

8. Troubleshooting

a. If no labelled DNA could be recovered, the labelling rate might have been too high and labelled DNA was lost during spin column purification (dyed spin column). In that case use less amounts of dye for click labelling.

b. Some standard commercial buffer components can decrease the click reaction efficiency or even impair reaction process. For example, TE buffer contains EDTA, which can chelate Cu^{II} ions and decrease the reaction rate. Thiol groups from reducing agents like β-mercaptoethanol or dithiothreitol (DTT) can stop the click reaction.

c. PCR fragments can have folded structures, which hinder accessibility of the functional groups that are needed for the click reaction. By adding some DMSO (5 - 10% (v/v) final) these folded structures are destabilized and improved click reaction progress can be observed.

d. Some dye-azide compounds show low solubility in the final reaction mixture, when used at higher concentrations and amounts, especially when high labeling rates are desired. This can impair reaction progress and decrease the yield. Try to use additional or alternative co-solvents like methanol or tetrahydrofuran if possible.

e. Some PCR target sequences with supercoiled and self-hybridizing structures might also cause problems. A prolonged denaturation step could help as well as the reduction of $MgCl_2$ content. Varying the $MgCl_2$ content leads to less specific primer binding but can help avoid unspecific fragments.