

ClickTech Oligo Link Kit - Oligo Oligo Click Ligation

This protocol was especially tested to link two oligonucleotides containing an alkyne and the other one an azide-modification without the need for splint oligos. Ligation and moreover purification is thus straight forward, fast and efficient.



The conjugation protocol herein was established using 70 pmol to 90 nmol of modified oligonucleotide and our proprietary solid catalyst (the “reactor”). As the reactor won’t be dissolved during the reaction, the handling of it is extremely easy, the reaction faster compared to traditional CuBr or CuSO₄ click chemistry reactions. Please be aware, that this protocol is only meant as a starting point. For other amounts and reaction partners used, please consider the [user manual](#) of the ClickTech Oligo Link Kits (BCK-OL) to obtain the optimal reaction outcome.

You will need following reagents and equipment:

- Alkyne-modified oligonucleotide and azide-modified oligonucleotide from enzymatic or commercial source, preferably dissolved in water
- ClickTech Oligo Link kits (BCK-OL)
- Microcentrifuge tubes
- Thermomixer, thermocycler or water bath
- Polyacrylamide or agarose gel electrophoresis
- Purification (e.g. ethanol precipitation, HPLC, purification kits...)
- Analytical HPLC system (optional)

Considerations:

- The “Reactor” contains a stable **heterogeneous catalyst**, which won’t be dissolved during the reaction.
- The click reaction can be performed with 10-100 μM DNA oligonucleotide solutions using this basic click protocol. For more concentrated samples a “preparative click” protocol might be needed. For RNA oligonucleotides check extra section in the User Manual.
- The click reaction is optimized for 1 h at 45 °C. For low concentrations (up to 20 μM) DNA decomposition in this reaction environment can start after 2 h at 45 °C. For optimal results it might be necessary to adjust the incubation time.
- Low reaction temperatures (e.g. 20 °C) can be applied as well in combination with longer reaction time.

- It is not feasible to use azide and alkyne functional groups within the same molecule when reaction to a second reaction partner is desired.
- Only terminal (containing CH on one end) alkynes can react with azides using the kit reaction conditions.

Click ligation procedure:

1. Add the appropriate amount of 10x Activator² to the Reactor, e.g. 2.5 μL Activator are added to Reactor 25 to be used with at a total reaction volume of 25 μL . Depending on Reactor and final volume, this needs to be adjusted (see table below & user manual).
2. Add an equimolar mixture of a singly modified alkyne- and a singly azide-modified oligonucleotide (same final concentration of each oligo) to dilute the Activator to a final 1x concentration.
Note: To achieve optimal reaction progress, do not increase or decrease the final activator amount. Oligo concentrations of 100 μM will give best yields; down to 10 μM good yields are achieved.
3. Close the vial and incubate the mixture at 45 $^{\circ}\text{C}$, 600 rpm for 1 h in a thermomixer. Alternatively, a water bath can be used. Make sure that the Reactor is within the reaction solution during the reaction. Spin down the solution if needed.
4. After the reaction: Spin down the Reactor. Transfer the supernatant with the product to a new vial.
Note: For long-term storage, reacted samples (without Reactor) should be kept at -20°C .
5. Analyze the reaction mixture by gel electrophoresis, HPLC or ion exchange chromatography (IEC). Purifications using column-based kits for oligonucleotide purification give good results. Make sure the length of the oligonucleotide is compatible with the purification kit. Alternatively, purification can be done by HPLC, IEC or ethanol precipitation (product loss likely).

Reaction Volumes and Amounts

This guide will help you to choose the Reactor and calculate the amounts needed.

Table: Reaction volumes, final concentrations and molar amounts in “basic” click reactions between oligonucleotides.

Reactor	10x Activator ²	Oligo solution ¹ V	Total V	Final oligo (c)	Oligo (n)
25	2.5 μL	22.5 μL	25 μL	10-100* μM	0.25-2.5 nmol
100	10.0 μL	90.0 μL	100 μL	10-100* μM	1.0-10 nmol

¹ Note: this “oligo solution” volume is referring to the final volume of the equimolar mixture of the singly-labeled azide and alkyne oligonucleotide to be used in the reaction.

* Note: In order to achieve a final oligo concentration of 100 μM , at least a 220 μM stock solution of each oligo is needed; for 10 μM , 22 μM is required.