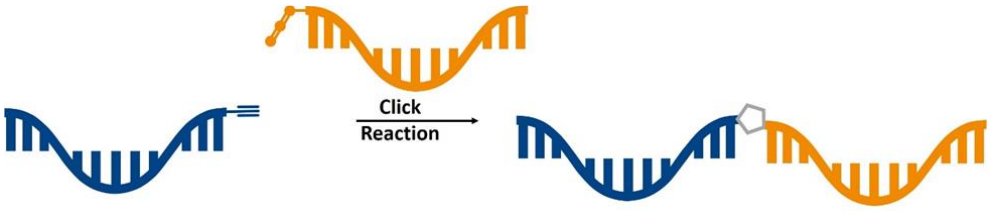
# Efficient Oligo – Oligo Click Ligation

This protocol was especially tested to link an oligonucleotide containing a terminal alkyne to an azide-modified oligonucleotide 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 $CuSO_4$ reactions. For the moment we are not selling the reactor separately, as it needs the special activator² coming in a ready-to-use kit system, but we want to share herein our preferred protocol. Soon we will also launch the products separately. 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 Oligo² Labeling Kits 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 (Baseclick will be glad to provide you with high quality oligonucleotides as well as labels; please check our website or inquire under info@baseclick.eu for an official quote)
- Oligo² Labeling kits (available in our shop)
- Microcentrifuge tubes
- Thermomixer, thermocycler or water bath
- Polyacrylamide or agarose gel electrophoresis
- Purification (e.g. ethanol precipitation, BaseClean kit, HPLC…)
- Analytical HPLC system (optional)

## Considerations:

- The "Reactor" contains a stable **heterogeneous catalyst**, which won't be dissolved during the reaction. **Do not** store the Reactor at –20°C, as it will lose functionality.
- 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 alkynes can react with azides using the kit reaction conditions.

## Click ligation procedure:

1. Add the appropriate amount of 10x Activator[2] 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 singly modified alkyne- and 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 °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. 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 (e.g. BaseClean kit) 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.