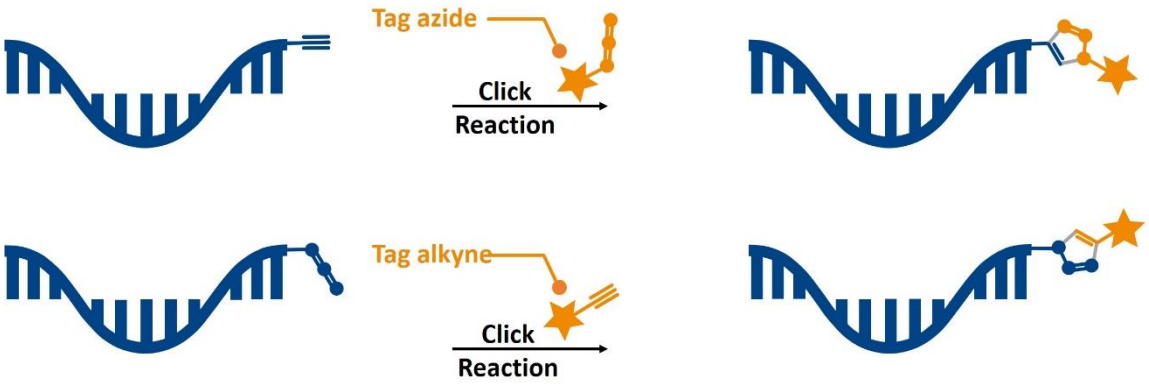
Oligo² - Dye Labeling Reactions

This protocol was especially tested for click chemistry with 70 pmol to 90 nmol of modified oligonucleotide using 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 and purification simplified. For the moment we are not selling the reactor separately, as it needs the special activator² coming in a ready-to-use kit system. We want anyhow to share herein our preferred protocol, so that you can get a feeling for the reaction. Please be aware, that this protocol is only meant as a starting point. Also, soon we will launch the products separately. 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- or azide-modified oligonucleotide (see custom oligo section)
- 10 mM solution of you preferred label-azide/ alkyne (see our click chemistry tools section)
- Eventually ethanol to dissolve your label-azide in case you bought is in solid form
- Oligo² Labeling kits (available in our shop)
- Reaction tubes (e.g. 1.5 mL vials)
- Table centrifuge
- Thermomixer
- Purification (e.g. ethanol precipitation, BaseClean kit, HPLC…)
- Analytical HPLC system

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.
- Only terminal alkynes can react with azides using the kit reaction conditions.

Click reaction procedure:

1. Dissolve/Dilute your label azide/label alkyne in DMSO or water to 0.2-10 mM to use it as a stock solution.
2. Add the appropriate amount of 10x Activator^2 to the Reactor, e.g. 2.5 µL 10x Activator^2 are added to Reactor 25 to be used at a total reaction volume of 25 µL. Depending on Reactor amount and final volume, this needs to be adjusted (see Table 1).
3. Add the alkyne/azide modified DNA oligonucleotide to the vial to a final concentration of 10-100 µM.
4. Add 2 equivalents of label azide/alkyne per equivalent of alkyne/azide in the oligonucleotide. For example, a 10 µM solution of a singly alkyne-modified oligonucleotide is mixed with 20 µM of a label azide for the click reaction.
5. 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. When using fluorophores, protect the vial from light. Make sure that the Reactor is within the reaction solution during the reaction. Spin down the solution if needed.
6. Spin down the Reactor. Transfer the supernatant with the clicked oligonucleotide to a new vial. **Note**: For long-term storage, reacted samples (without Reactor) should be kept at –20 °C.
7. Analyze the reaction mixture by gel electrophoresis, HPLC or ion exchange chromatography (IEC). Purifications using column-based kits for oligonucleotide purification (e.g. PCR purification kit from Qiagen) 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).

Exemplary Label-Oligo Click

This guide will help you decide which stock solution concentration of the label azide/alkyne should be prepared. All concentrations within the table refer to the stock solution concentrations for exemplary setups using two equivalents of label azide/alkyne for a singly modified oligonucleotide alkyne/azide.

Table 1: Exemplary volumes needed for reaction setups of "basic" label-oligonucleotide click reactions.

Reactor	V (Activator^2)	c (Oligo)	V (Oligo)	c (Label)	V (Label)	V (H_2O)	Oligo (n)
25	2.5 µL	10 µm	20.0 µL	200 µM	2.0 µL	0.5 µL	200 pmol
25	2.5 µL	50 µm	20.0 µL	1 mM	2.0 µL	0.5 µL	1.0 nmol
25	2.5 µL	100 µm	20.0 µL	2 mM	2.0 µL	0.5 µL	2.0 nmol
100	10.0 µL	10 µm	80.0 µL	1 mM	1.6 µL	8.4 µL	0.8 nmol
100	10.0 µL	50 µm	80.0 µL	2 mM	4.0 µL	6.0 µL	4.0 nmol
100	10.0 µL	100 µm	80.0 µL	10 mM	1.6 µL	8.4 µL	8.0 nmol