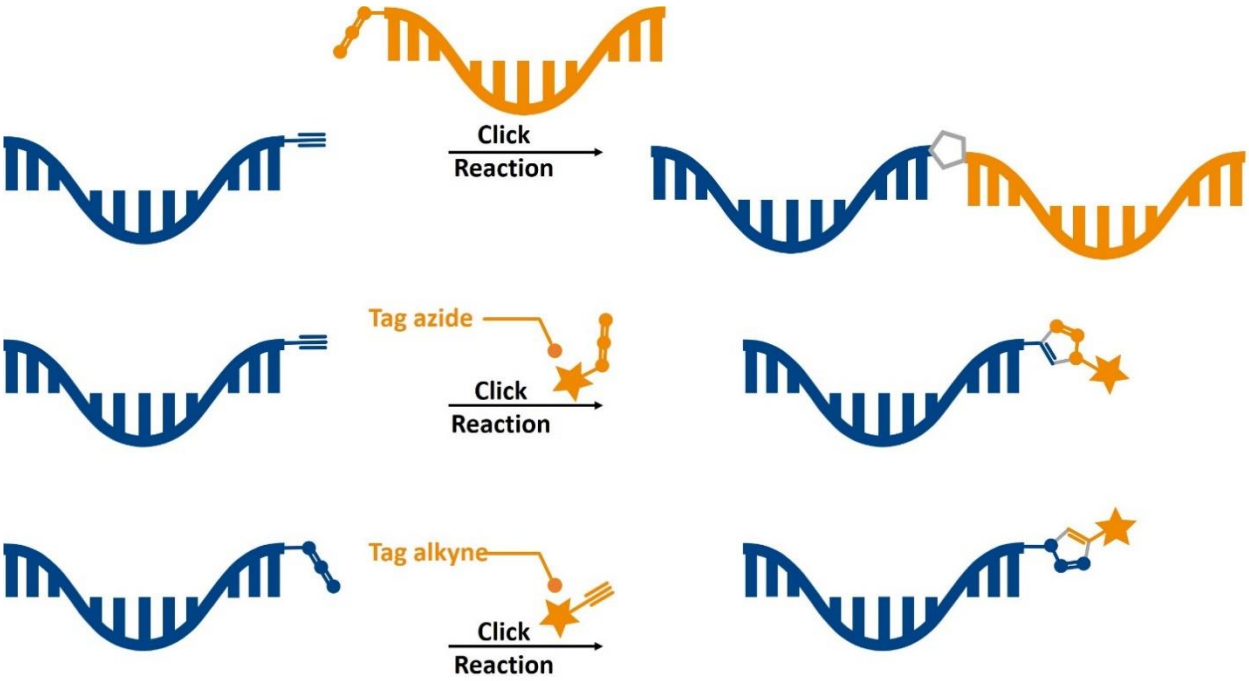
User Manual

ClickTech Oligo Link Kit



Version 1.1

Ordering information

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

ClickTech Oligo Link Kit:

Product Number	Product Name	Amount Range of Oligonucleotide per Reaction[1]
BCK-OL-S	ClickTech Oligo Link Kit S	From 70 pmol to 22 nmol
BCK-OL-L	ClickTech Oligo Link Kit L	From 1 nmol to 90 nmol

1 = These are the minimal and maximal amounts of reacted oligonucleotide that can be achieved using the kits. The actual feasible amounts are also dependent on the available concentrations of the reaction partners.

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

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ClickTech Oligo Link Kit

For click chemistry with 70 pmol to 90 nmol of modified oligonucleotide

Introduction and product description:

This kit was especially optimized to link an oligonucleotide containing a terminal alkyne to an azide-modified oligonucleotide without the need for splint oligos. Labeling of alkyne- or azide-modified oligonucleotides using label-azides or label-alkynes, respectively, is also possible using this kit.

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Please read the material safety data sheets (MSDS) provided for each product/component available online at www.baseclick.eu.

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

1. Materials and Storage Conditions

Table 1: Contents of the ClickTech Oligo Link Kits and storage conditions.

Color code	Quantity	Component	Storage
yellow	50 µL (Kit S) ---------- 150 µL (Kit L)	10x Activator²	RT °C
green	10x	Reactor 25 (Kit S) ---------- Reactor 100 (Kit L)	RT
blue	1 mL	DMSO (solvent)	RT

2. Required Material and Equipment – not provided with this kit

- Alkyne-modified oligonucleotide and azide-modified oligonucleotide from enzymatic or commercial source, preferably dissolved in water (read also section **3** and **11**).
- In case of labeling reactions: label-azides or label-alkynes (depending on oligonucleotide functionality)
- baseclick will be glad to provide you with high quality oligonucleotides as well as labels; please check our website or inquire *via* email at quotes@baseclick.eu for an official quote.
- Microcentrifuge tubes
- Thermomixer, thermocycler or water bath
- Polyacrylamide or agarose gel electrophoresis
- PCR or gel purification kit (optional, e.g. from Qiagen)
- HPLC/IEC system for analyses (optional)

3. General Considerations

- The "Reactor" contains a stable **heterogeneous catalyst**, which won't be dissolved during the reaction.
- The DMSO component is not needed for the standard protocol, but is included in the kit to dissolve label azides/alkynes or as additive (see section **11**).
- The click reaction can be performed with 10-100 µM DNA oligonucleotide solutions using the basic click protocol. For more concentrated samples a "preparative click" protocol might be needed. For RNA oligonucleotides check extra section.
- 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.
- Only terminal alkynes can react with azides using the kit reaction conditions.
- It is not feasible to use azide and alkyne functional groups within the same molecule when reaction to a second reaction partner is desired.
- Read section **11** (Troubleshooting) before getting started.

4. Basic Label-Oligo Click Protocol

4.1. Dissolve/Dilute your label azide/label alkyne in DMSO or water to 0.2 - 10 mM to use it as a stock solution.

4.2. 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 2**).

4.3. Add the alkyne/azide modified DNA oligonucleotide to the vial to a final concentration of 10 - 100 µM.

Note: For reactions of more concentrated solutions, see Preparative Label-Oligo Click Protocol on page **7**.

4.4. 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.

4.5. 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.

4.6. 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.

4.7. 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 2: 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

5. Basic Oligo Link Click Protocol (Oligo – Oligo Connection)

5.1. 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 3**).

5.2. 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. For reaction of more concentrated solutions see “preparative clicks” on page **8**.

5.3. 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.

5.4. 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.

5.5. 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).

Reaction Volumes and Amounts

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

Table 3: 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.

6. Preparative Label-Oligo Click Protocol

For click reactions using more concentrated oligo stock solutions **(>100 µM final concentration)** the protocol needs some adaption.

6.1. Dissolve/Dilute your label azide/label alkyne in DMSO or water to 10 - 50 mM to use it as a stock solution.

6.2. Add the appropriate amount of 10x Activator[2] to the Reactor, e.g. 10 µL Activator[2] are added to Reactor 100 to be used with a total reaction volume of 100 µL. Depending on Reactor and final volume, this needs to be adjusted (see **Table 4**).

6.3. Add the alkyne/azide modified oligonucleotide to the vial to a final concentration of 100 - 700 µM.

6.4. Add 2 - 4 equivalents of label azide/alkyne per equivalent of alkyne/azide in the oligonucleotide. For example a 200 µM solution of a singly alkyne-modified oligonucleotide is mixed with 400 - 800 µM of a label azide for the click reaction.

6.5. Incubate the mixture at 45 °C, 600 rpm for 2 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.

6.6. Spin down the Reactor. Transfer the supernatant with the product to a new vial. Wash the remaining pellet with half the reaction volume of H_2O to get all reaction mix to the new vial.

Note: For long-term storage, reacted samples (without Reactor) should be kept at – 20 °C.

6.7. 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 Preparative Label-Oligo Click Setups

Table 4: Exemplary volumes needed for reaction setups of "preparative" label-oligonucleotide clicks.

Reactor	V (Activator[2])	C[1] (Oligo)	V(Oligo)	C (Label)	V[2] (Label)	V (H_2O)	Oligo (n)
25	2.5 µL	200 µM	20 µL	10 mM	1.2 µL	1.3 µL	4.0 nmol
25	2.5 µL	500 µM	20 µL	20 mM	1.5 µL	1.0 µL	10 nmol
25	2.5 µL	1.0 mM	20 µL	50 mM	1.2 µL	1.3 µL	20 nmol
100	10 µL	200 µM	80 µL	10 mM	4.8 µL	5.2 µL	16 nmol
100	10 µL	500 µM	80 µL	20 mM	6.0 µL	4.0 µL	40 nmol
100	10 µL	1.0 mM	80 µL	50 mM	4.8 µL	5.2 µL	80 nmol

[1] Concentration of the oligonucleotide stock solution.

[2] Note: The volume of label azide/alkyne in the table has been calculated using 3 equivalents for the reaction.

7. Preparative Oligo Link Click Protocol (Oligo – Oligo Connection)

For click reactions between a singly modified alkyne oligonucleotide and a singly modified azide oligonucleotide, using more concentrated oligo stock solutions **(>200 µM stock, >100 µM final concentration)** the protocol needs some adaption compared to the basic protocol described on page **6**.

7.1. Dissolve/Dilute your oligonucleotide azide/alkyne in water to 200 µM - 2.0 mM to use it as a stock solution. The same stock solution concentration of each alkyne and azide oligo is recommended to allow convenient reaction setup.

Note: The very common TE buffer, which is commonly used for DNA, is not suitable for click reactions involving this kit, since the EDTA content will interfere with reaction catalysis (see also section **11**).

7.2. Add the appropriate amount of 10x Activator² to the Reactor, e.g. 10 µL Activator² are added to Reactor 100 to be used with a total reaction volume of 100 µL. Depending on Reactor and final volume, this needs to be adjusted (see **Table 5**).

7.3. Add the alkyne/azide modified oligonucleotide to the vial to a final concentration of 100 - 900 µM. Equimolar amounts of each reaction partner are recommended, i.e. singly modified oligonucleotides with a stock solution of the same concentration will be applied at same volumes (see **Table 5** for examples). 90% of the total reaction volume can be used for the reaction partners, dH_2O can be used to reach the total volume if necessary. Do not exceed or decrease the total reaction volume to obtain good reaction progress.

7.4. Incubate the mixture at 45 °C, 600 rpm for 2 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.

7.5. Spin down the Reactor. Transfer the supernatant to a new vial. Wash the remaining pellet with half the reaction volume of H_2O to get all reaction mix to the new vial.

Note: For long-term storage, reacted samples (without Reactor) should be kept at – 20 °C.

7.6. 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 Preparative Oligo Link Click Setups

Table 5: Exemplary volumes needed for reaction setups of "preparative" oligonucleotide-oligonucleotide clicks.

Reactor	V (Activator²)	c (Oligo1)	V (Oligo1)	c (Oligo2)	V (Oligo2)	c (final)	Oligo (n)
25	2.5 µL	500 µM	11.2 µL	500 µM	11.2 µL	224 µM	5.6 nmol
25	2.5 µL	1.0 mM	11.2 µL	1.0 mM	11.2 µL	448 µM	11 nmol
25	2.5 µL	2.0 mM	11.2 µL	2.0 mM	11.2 µL	896 µM	22 nmol
100	10 µL	500 µM	45 µL	500 µM	45 µL	225 µM	23 nmol
100	10 µL	1.0 mM	45 µL	1.0 mM	45 µL	450 µM	45 nmol
100	10 µL	2.0 mM	45 µL	2.0 mM	45 µL	900 µM	90 nmol

oligo1 = e.g. singly alkyne modified oligonucleotide

oligo2 = e.g. singly azide modified oligonucleotide.

8. Special Protocols

8.1. Smallest Possible Click Reaction

In order to minimize the amounts needed for a single click reaction, it is possible to adjust the click protocol for smallest scale reactions. When e.g. using 100 µM final oligonucleotide concentrations, only 700 pmol oligonucleotide is needed for a single click. Down to 70 pmol oligonucleotide per reaction can be used without a major decrease in yield.

8.1.1. A single **Reactor** pellet (0.6 - 0.8 mm diameter) is transferred to a 200 µL vial.

8.1.2. Add 0.7 µL of 10x Activator² to the Reactor pellet.

8.1.3. A volume of 6.3 µL can be used for the reaction partner mix, e.g. alkyne containing oligonucleotide and fluorophore azide.

8.1.4. Briefly spin down the reaction mix and control if the Reactor pellet is in solution.

8.1.5. Incubate the reaction mix at 45 °C for 45 min without shaking. Spin down the Reactor. Transfer the supernatant to a new vial.

Note: For long-term storage, reacted samples (without Reactor) should be kept at – 20 °C.

8.1.6. 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).

8.2. RNA Oligonucleotide Click Reactions

Click reactions involving alkyne and azide modified RNA oligonucleotides have been performed successfully using this ClickTech Oligo Link Kit protocol. RNA is more prone to chemical and enzymatic degradation than DNA, please handle with precautions. We recommend to use RNase free components for the reactions and to keep the RNA on ice apart from the reaction incubation step. The provided reagents in this kit are not tested to be RNase free.

8.3. Small Molecule Click Reactions

The ClickTech Oligo Link Kit S protocol has been successfully used to join azide modified molecules with terminal alkyne containing small molecules. For example, 1.1 mM of an amino alkyne linker (BCL-091) was clicked to 1.1 mM 6-FAM azide (BCFA-001) in 2 hours at 45 °C. Complete conversion of the 6-FAM azide was observed by analytical HPLC. Using the same click conditions, reactions of amino alkyne linker with 3-Azido-7-hydroxycoumarin (BCFA-047), Eterneon-Red 645 Azide (BCFA-201) and 5/6-Sulforhodamine 101 PEG3-Azide (BCFA-044) proceeded with at least 91% conversion yield.

Please note that these reaction examples are no guarantee that every reactant combination between azide and alkyne containing small molecules will perform well using the ClickTech Oligo Link Kit. It might be a possible option for reaction catalysis. Experimental optimization of reaction time and temperature for each system should be considered.

9. Alternative Reaction Temperatures

In case that 45 °C incubation is not possible, alternative reaction temperatures can be used. This will require adjustment of the incubation time. Here are our recommendations for some alternative reaction temperatures and the standard condition in the basic protocol (10 - 100 µM oligo) for standard click ligation reactions described herein. If more complex (bio)molecules should be clicked to oligonucleotides tests on finding the optimal conditions need to be performed using these recommendations as a starting point.

Temperature	Duration
20 °C	4 - 5 h
30 °C	90 min
45 °C	60 min
60 °C	50 min

10. Exemplary Gel Image from Oligo link Clicks

Here is an example where the kit has been used to click ligate a 5'-azide oligo (59 mer) to a 5'-alkyne oligo (59 mer) using varying oligonucleotide amounts. Very low oligonucleotide amounts were studied in order to determine the lower working range for the kit. No splint oligonucleotides were used for preorganization of the strands.

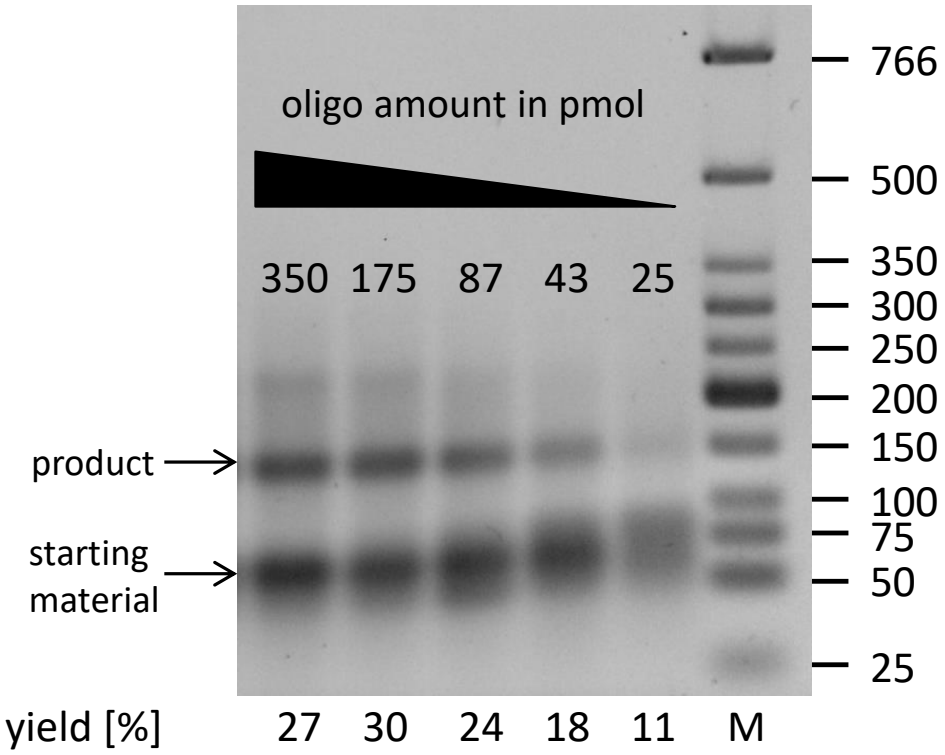

Figure 1. Ethidium bromide stained 3% agarose gel from oligo-oligo click reactions using different oligo amounts. For the analysis the same amount of oligonucleotide was loaded onto each lane of the gel. The sample volumes were adjusted accordingly. Yield means the densidometric ratio between product band and the sum of all bands in the corresponding lane.

11. Trouble Shooting

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.

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

Some (dye) labels show low solubility in the final reaction mixture, when used at higher concentrations and amounts, especially in the preparative label-oligo click protocol. This can impair reaction progress and decrease the yield. Try to use additional or alternative co-solvents like methanol or tetrahydrofuran if possible.

When slow reaction progress is observed, increasing the reaction temperature, e.g. to 60 °C might help to improve the reaction (see **9**) and solubility of the reaction partners.

Dissolved oxygen from air in stock solutions can change the reaction kinetic. Much oxygen decreases, less oxygen increases the reaction progress. As cold solutions can keep more dissolved gas, cold stock solutions contribute the most oxygen. A main source for the change of dissolved oxygen are freezing and thawing cycles. By freshly dissolving dry components in degassed or room temperature stored water the reaction progress can increase. Saturation of solutions with inert gas (e.g. N_2 or argon) can also increase the reaction progress, if available. Be aware that this will not only decrease the reaction time, but might also decrease the time from which oligonucleotide degradation starts.