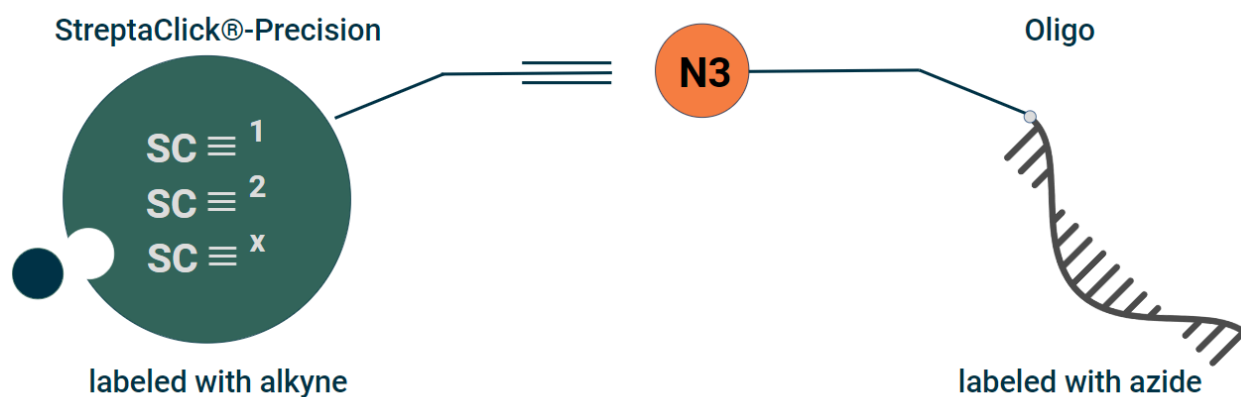


Protocol to click Oligonucleotides to StreptaClick®-Precision

StreptaClick®-Precision is a form of monovalent streptavidin with affinity equivalent to that of the tetravalent that also carries a predefined number of alkyne groups that allows the precise conjugation of any azide-carrying molecule.



The following protocol describes the procedure to react an oligo with a single azide group with 0.5 mg of StreptaClick®-Precision^{≡1} (10 μM) with a single alkyne group.

- StreptaClick®-Precision^{≡1} is provided as 10 μM, in 20 mM Tris-HCl pH 8.0, 50 mM NaCl
- Dissolve your azide-modified oligonucleotide in the appropriate amount of water or TE buffer to obtain 1mM stock solution
- Solution A, stock 50x: 5 mM CuSO₄, 25 mM THPTA, 50 mM AG-HCl
- Solution B, stock 50x: 50 mM Ascorbic acid – *prepare fresh before each reaction!* –

Abbreviations:

Tris(benzyltriazolylmethyl)amine → THPTA

Aminoguanidine Hydrochloride → AG-HCl

Mix in the following order for 960 µl reaction:

1. 910 µl StreptaClick®-Precision≡¹ with 1 alkyne
2. 20 µl Solution A
3. 10 µl Oligonucleotide with 1 azide (1.1x molar excess)
4. Vortex slightly and purge nitrogen for 30 sec (if possible)
5. 20 µl Solution B
6. Vortex slightly and purge nitrogen for 30 sec (if possible)
7. Incubate overnight (under slight rotation or shaking if possible)

If you want to use other StreptaClick®-Precision≡^x versions with more than 1 alkyne groups, just increase the reaction volume up to 990µl by adding more oligonucleotide on step 3 as follows:

- StreptaClick®-Precision≡¹ → 10 µl Oligo
- StreptaClick®-Precision≡² → 20 µl Oligo
- StreptaClick®-Precision≡³ → 30 µl Oligo
- StreptaClick®-Precision≡⁴ → 40 µl Oligo

You can use the oligo-conjugated StreptaClick® directly if the reaction components do not interfere with the downstream application (oligo molar excess is only 1.1, and most of it is consumed during the CuAAC reaction). If you need to purify the reaction, you can use 10-30 kDa MWCO spin columns or (depending the oligo size and nature) or a purification method like ion exchange chromatography.