Manual

Click chemistry conjugation of oligonucleotides to StreptaClick® Precision using Strain-Promoted Azide-Alkyne Click Chemistry reaction (SPAAC)

Kit contents and Storage:

StreptaClick® Precision Azide/DBCO (lyophilized): -20°C
1X SPAAC reaction buffer (1 ml): 4°C to 25°C
100X Tris-EDTA (TE) Buffer (1 ml): 4°C to 25°C

Not provided in the kit

Microcentrifuge tube
Spin columns

Applications

This manual describes conjugation of oligonucleotides to StreptaClick® Precision. StreptaClick® Precision can also be used for conjugation of other molecules, such as lipid nanoparticles and fluorochromes.

General information

StreptaClick® Precision is a variant of tetrameric streptavidin that binds monovalently to biotinylated antibodies with high binding affinity. This protocol outlines the procedure for conjugation of oligonucleotides to StreptaClick® Precision using Strain-Promoted Azide-Alkyne Click Chemistry reaction (SPAAC), a copper-free version of click chemistry.

CONJUGATION PROTOCOL

1. Allow the lyophilized StreptaClick® Precision to reach room temperature. Reconstitute to a final concentration of 5 mg/mL (91 µM) with the provided 1x SPAAC reaction buffer. For 0.1 mg StreptaClick® Precision add 20 µl buffer; for 0.5 mg, add 100 µl buffer; for 2 mg, add 400 µl buffer. Reconstituted StreptaClick® Precision that is not used at once can be aliquoted and stored at -20°C.

2. Mix StreptaClick® Precision with 1.3-fold molar excess of oligonucleotide according to Table 1 below. *For example, to conjugate 0.1 mg StreptaClick® Precision, mix 20 µl StreptaClick® Precision (5 mg/ml) with 12 µl of 200 µM oligonucleotide solution.*

3. Incubate at room temperature overnight.

4. Dilute the reaction mixture with 1X Tris EDTA Buffer according to Table 1. The final StreptaClick® Precision concentration is 1.25 mg/ml (22.6 µM) and the final buffer is 1X Tris EDTA, pH8, 150 mM NaCl.

Table 1.

Reaction size (mg SC® Precision)	SC® Precision (5 mg/ml, 91uM)	Oligonucleotide (200 uM / 100 uM)	1x TE Buffer (1:100 from 100x TE Buffer)
0.1 mg →	20 µl	12 µl (200 uM)	48 µl
		24 µl (100 uM)	36 µl
0.5 mg →	100 µl	60 µl (200 uM)	240 µl
		120 µl (100 uM)	180 µl
2.0 mg →	400 µl	240 µl (200 uM)	960 µl
		480 µl (100 uM)	720 µl

Removal of unconjugated oligonucleotides

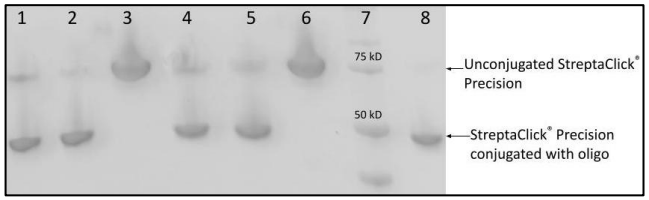
For removal of excess oligonucleotides, utilize a 30 kDa MWCO spin column (for oligonucleotides <20 kDa).

Removal of unconjugated StreptaClick® Precision

For large DNA constructs (>60 kDa) a 100 kDa MWCO spin column can be used to wash out unconjugated StreptaClick® Precision. For large and small DNA constructs, ion exchange chromatography can be used to separate conjugated and unconjugated StreptaClick® Precision.

Assessment of reaction completion

The degree of StreptaClick® Precision conjugation is 80-100% for most oligonucleotides. The conjugation product can be checked using native polyacrylamide gel electrophoresis (PAGE). Load samples without heating and without reducing agents. Use standard running buffer, for example 25 mM Tris, 192 mM glycine and 0.1% SDS pH 8.3.



1. StreptaClick® Precision – Azide + 20 bp oligo – DBCO
2. StreptaClick® Precision – Azide + 20 bp oligo – DBCO
3. Control: StreptaClick® Precision – Azide
4. StreptaClick® Precision – DBCO + 43 bp oligo – azide
5. StreptaClick® Precision – DBCO + 41 bp oligo – azide
6. Control: StreptaClick® Precision – DBCO
7. Marker
8. StreptaClick® Precision – DBCO + 37 bp oligo – azide

Trouble shooting

Low degree of conjugation

- Increase the molar excess of oligonucleotides in the conjugation reaction.
- Perform the conjugation reaction under shaking conditions.
- Use fresh DBCO-oligonucleotides/StreptaClick® Precision DBCO. DBCO loses its reactivity over time due to oxidation. Low yield of conjugated product may be due to degraded DBCO.