Manual

StreptaClick® Precision

KROMNIGON
Multiplex IHC made easy

Size:

StreptaClick® Precision – Alkyne or Azide or Oligo
0.1 mg or 0.5 mg or 2 mg

Time:

1 hour

Content and Storage:

StreptaClick®Precision +4°C
(provided in 20 mM Tris-HCl buffer, pH 8.0)

General information

StreptaClick® Precision is developed for site-specific conjugation with a defined number of attachment groups. Furthermore, StreptaClick® Precision has a unique design with maximum separation of the sites for conjugation and biotin binding. This design makes StreptaClick® Precision ideal for attachment of oligonucleotides and other bulky molecules without affecting the biotin binding quality and without causing aggregation when mixed with biotinylated proteins.

StreptaClick® Precision is currently provided with a single azide or alkyne group for standard click chemistry. It is also available pre-conjugated with a single 14 mer oligo or a single bright fluorochrome (488 and 647).

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 0.1 mg (1.8 nmole) StreptaClick® Precision – Azide/Alkyne, using CuAAC chemistry.

Abbreviations

THPTA: Tris(benzyltriazolylmethyl)amine

AG-HCl: Aminoguanidine Hydrochloride

(A) Preparation

1. Dissolve your azide/alkyne-modified oligonucleotide in the appropriate amount of water or TE buffer
2. Prepare Solution A, stock 50x: 5 mM CuSO 4 , 25 mM THPTA, 50 mM AG-HCl (can be stored at +4°C)
3. Prepare Solution B, stock 50x: 50 mM Ascorbic acid in MQ water. Must be prepared fresh!

(B) Click Reaction

In tube A:

1. Add 50 µl of a 2 mg/ml StreptaClick® Precision solution, or 250 µl of a 0.5 mg/ml StreptaClick® Precision solution.

2. Add 1.1x molar excess of oligonucleotide-alkyne/azide.

3. Make up volume to 288 µl with PBS.

In tube B:

1. Mix 6 µl of Solution A with 6 µl of Solution B.

2. Transfer the 288 µl from tube A to tube B and mix by pipetting up and down.

3. Purge with nitrogen for 30 sec (if possible) and seal with parafilm.

4. Incubate under rotation/shaking (if possible) at RT for 1-2 hours or at +4°C overnight

If you need to purify the reaction, use 10-30 kDa MWCO spin columns or (depending on the oligo size and nature) or a purification method like ion exchange chromatography.

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