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A Geno Technology, Inc. (USA) brand name

Sodium Cyanoborohydride

A Reducing Agent for Stable Bonds Between Aldehyde and Amine Groups

(Cat. # 786-061, 786-062)

INTRODUCTION

Carbonyl groups, including aldehydes, ketones and glyoxals, react with amines to form Schiff base intermediates that are in equilibrium with their free forms. The labile Schiff's base interaction can be stabilized by chemical reduction. If the Schiff's base is formed between an aldehyde and an amine then sodium cyanoborohydride is routinely used for the reduction of the Schiff's base to a covalent bond[1] (figure 1).

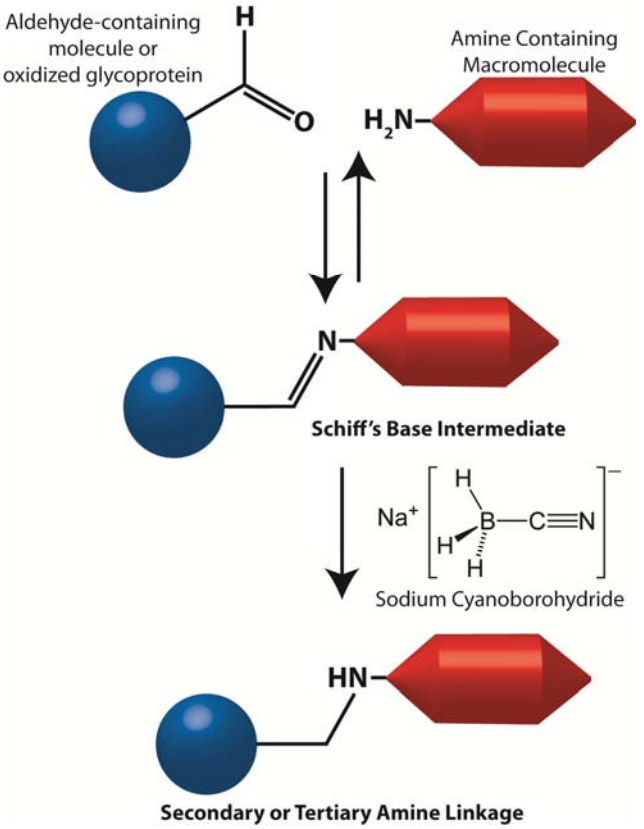


Figure 1

Sodium cyanoborohydride is preferred as a reducing agent over sodium borohydride as the latter will also reduce the reactive aldehydes to hydroxyls at the same time as reducing the Schiff's bases. The cyanoborohydride offers five times milder reduction compared to borohydride in the reductive amination process, reducing the Schiff's bases, but not the aldehydes[2].

The reductive amination process is pH-dependent, being more efficient in acidic conditions, especially at high pH.

ITEM(S) SUPPLIED

Description	Cat. # 786-061	Cat. # 786-062
Sodium Cyanoborohydride ($NaCNBH_3$)	0.5g	4 x 0.5g

STORAGE CONDITIONS

This product is shipped at ambient temperature. Upon receipt store desiccated at room temperature.

WARNING

Sodium cyanoborohydride is highly toxic and must be prepared and used in a fume hood. See the Safety Data Sheet (SDS) for further information.

PROPERTIES

- Synonym: Sodium cyanotrihydridoborate
- Linear formula: $NaCNBH_3$
- CAS #. 25895-60-7
- Molecular weight: 62.84
- Form: White to yellow crystalline powder

PROTOCOL FOR COUPLING GLYCOPROTEIN TO AMINE GROUPS

Additional Item(s) Required

- Oxidation Buffer: We recommend our Optimizer Buffer™ V (Cat. # BKC-08) or 0.1M sodium acetate, pH 5.5. Neutral pH buffers, such as PBS, can be used but are less efficient than then slightly acidic conditions. Avoid buffers with primary amines, such as Tris or glycine, or sugars as these will compete in the reaction.
- Sodium metaperiodate (Cat. # BKC-15)
- Desalting columns. *SpinOUT™ GT-600, 5ml, Cat. # 786-704*
- 1X PBS
- 1N Sodium hydroxide
- Blocking buffer: 1M Tris.HCl, pH 7.4 or 1M Ethanolamine, pH9.6

Glycoprotein Oxidization

1. Dissolve 0.5-10mg glycoprotein in 1ml Oxidation Buffer.
2. Add 2mg sodium metaperiodate to an amber vial. Using 2mg for each 1ml protein solution results in ~10mM sodium metaperiodate. Add the protein solution to the amber vial and swirl to dissolve the sodium metaperiodate.
3. Alternatively, dissolve 4.3mg sodium metaperiodate in 1ml oxidation buffer and then add 1ml to every 1ml glycoprotein solution.
   NOTE: *The steps involving sodium metaperiodate are light sensitive and must be performed in an amber vial.*
   NOTE: *To only oxidize the sialic groups, use 1mM final concentration of sodium*

periodate by adding 50µl 20mM sodium metaperiodate to every 1ml glycoprotein solution.

4. Incubate at room temperature for 30 minutes.

Remove Sodium Meta-Periodate (Oxidizing Reagent) with SpinOUT GT-600, 5ml

1. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.
2. Prepare the SpinOUT™ column by centrifuging the SpinOUT™ columns at 1,000g for 1 minute to compact the resin.
3. Remove the top and then bottom caps. Place into an appropriate collection tube.
4. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.
5. Equilibrate the column with 1X PBS by applying at least 5 column volumes of buffer in batches of 1-2 column volumes. Centrifuge the column at 1,000g for 2 minutes to remove the buffer after each application.
6. Place the column in a new collection tube and remove the cap.
7. Slowly, apply the protein solution to the center of the SpinOUT™ resin.
8. Centrifuge the column at 1,000g for 8 minutes to collect the desalted protein solution. Discard the column.

Conjugate Oxidized Glycoprotein to Amine or Hydrazide Containing Molecule

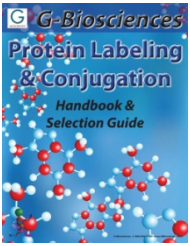
1. Prepare the sodium cyanoborohydride an hour before it is required below. Dissolve 160mg in 0.5ml 1N NaOH to generate a 5M stock solution.
   CAUTION: *Sodium cyanoborohydride is toxic, prepare in a fume hood.*
2. Dissolve the amine or hydrazide containing molecule (protein) in PBS at a concentration of 10mg/ml.
   NOTE: *If the molecule is in an amine containing buffer, such as Tris or glycine, dialyze or desalt against PBS before use.*
3. Combine the oxidized and desalted glycoprotein with the amine or hydrazide containing molecule in suitable amounts to give the desired molar ratio. We recommend 4 to 15 molar excess of amine or hydrazide containing molecule over oxidized glycoprotein.
4. Add 10µl 5M sodium cyanoborohydride solution for every 1ml conjugation mixture.
5. Incubate at room temperature for 4-6 hours or at 4°C overnight.
6. Add 50µl Blocking buffer for every ml of conjugation mixture to block non-reacted aldehyde sites and incubate at room temperature for 30 minutes.
7. Purify conjugated protein by desalting or dialysis. We recommend our SpinOUT™ desalting columns or Tube-O-DIALYZER™ devices (Cat. # 786-610 to 786-624).

REFERENCES

1. Hermanson, G (1996) Bioconjugate Techniques, Academic Press
2. Peng, L. et al (1987) Appl. Biochem. Biotechnol. 14:91

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



http://info.gbiosciences.com/complete-protein-labeling-conjugation-handbook/

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