

# AminoLink™ Reductant

(sodium cyanoborohydride, NaCNBH<sub>3</sub>)

44892

1291.3

**Number****Description**

44892

**AminoLink Reductant** (sodium cyanoborohydride, NaCNBH<sub>3</sub>), 2 × 1g

Form: white to yellow crystalline powder; may yield slightly hazy solution in water

Molecular Weight: 62.84

CAS # 25895-60-7

**Caution:** NaCNBH<sub>3</sub> is toxic and must be used in a fume hood. See the Material Safety Data Sheet (MSDS) for additional information.

**Storage:** Upon receipt store desiccated at room temperature. Product shipped at ambient temperature.

**Introduction**

Thermo Scientific AminoLink Reductant promotes the formation of stable bonds between aldehyde- and amine-containing molecules, enabling efficient labeling, conjugation and immobilization of proteins and other research molecules. Carbonyl groups such as aldehydes, ketones and glyoxals react spontaneously with amino groups to form Schiff base intermediates that are in equilibrium with their free forms. The interaction is pH-dependent, being somewhat more efficient in acidic conditions and especially strong at high pH. Addition of AminoLink Reductant (sodium cyanoborohydride) to a reaction in which Schiff base formation has occurred results in complete reduction of the labile Schiff base intermediate to a chemically stable bond<sup>1</sup> (Figure 1). Unlike sodium borohydride, sodium cyanoborohydride is sufficiently mild to avoid adversely reducing aldehydes to nonreactive hydroxyls.

The overall reaction of carbonyl groups to primary amino groups using sodium cyanoborohydride is called reductive amination. Immobilization by reductive amination of amine-containing biological molecules onto aldehyde-containing solid supports is used to create matrices for affinity purification of antibodies and other molecules. For detailed immobilization procedures, consult the instructions for AminoLink Plus Coupling Resin or the AminoLink Plus Immobilization Kit (see Related Thermo Scientific Products).

The example protocol presented in these instructions describes conjugation of a glycoprotein to amine or hydrazide derivatives. To conjugate glycoproteins by reductive amination, sugars in the polysaccharide groups must be oxidized to create reactive aldehyde groups using sodium *meta*-periodate. Treatment of glycoproteins with sodium *meta*-periodate generally creates sufficient aldehyde groups to be used as targets for efficient conjugation with amine- or hydrazide-containing molecules, including other proteins, biotin reagents and fluorescent labels (see Related Thermo Scientific Products). Because glycoproteins contain their own primary amino groups, polymerization of an oxidized protein may occur unless a sufficient molar excess of the intended amine-containing target molecule is added to the reaction.

Reductive amination using AminoLink Reductant is an extremely flexible and reliable method for many kinds of conjugation experiments besides those mentioned above and described in detail in these instructions. For example, where polymerization of a protein or small amine-containing molecule is the goal, one may use aldehyde-activated dextran (see Related Thermo Scientific Products) or other oxidized pure polysaccharide as a backbone for conjugating multiple amine-containing molecules.

## Example Protocol: Conjugation of Glycoprotein to Amine or Hydrazide Derivatives<sup>2,3,4</sup>

### Additional Materials Required

- Sodium *meta*-Periodate (Product No. 20504)
- Desalting Columns (e.g., Product No. 89891)
- Phosphate Buffered Saline (PBS; Product No. 28372): 0.1M phosphate, 0.15M NaCl, pH 7.2. Other buffers may be used if they do not contain either carbohydrates or primary amines (e.g., do not use Tris buffer).
- 1mL of 1N sodium hydroxide (NaOH) to make AminoLink Reductant stock solution
- Blocking Buffer: 1.0M Tris•HCl, pH 7.4 or 1.0M ethanolamine, pH 9.6

### A. Oxidize Glycoprotein to Create Reactive Aldehyde Groups

**Note:** Steps 2 and 3 of this preparation are light-sensitive and must be performed in an amber vial.

1. Dissolve 0.5-10mg of glycoprotein or polyclonal antibody in 1mL of PBS.

**Note:** Most polyclonal antibodies are glycosylated, but most monoclonal antibodies are not. Only glycosylated proteins can be oxidized to produce reactive aldehydes for successful coupling by reductive amination. If uncertain about the glycosylation status of the antibody or protein, use a carbohydrate estimation kit (see Related Thermo Scientific Products).

2. Weigh 2mg of sodium *meta*-periodate into an amber vial. Use 2mg for each milliliter of protein solution; this quantity results in ~10mM sodium *meta*-periodate, which is sufficient for general carbohydrate oxidization without undue risk of oxidizing amino acids.
3. Add the glycoprotein solution to the vial containing sodium *meta*-periodate; gently swirl vial until the powder dissolves.
4. Incubate the oxidation reaction for 30 minutes at room temperature.

### B. Desalt to Remove Oxidizing Agent

1. Equilibrate desalting column with PBS.
2. Apply the oxidized glycoprotein sample to the desalting column and allow it to enter the gel bed.  
**Note:** For best results, use a sample volume < 20% of the volume of the desalting column gel bed.
3. Add PBS to the column and collect separate 0.5mL fractions as they emerge from the column.
4. Identify the fractions containing protein by measuring for those having peak absorbance at 280nm. The fractions corresponding to the first absorbance peak are those containing the oxidized protein.

### C. Conjugate Amine- or Hydrazide-containing Molecule to Oxidized Glycoprotein

1. An hour before use in Step 4, dissolve 160mg of AminoLink Reductant in 0.5mL of 1M NaOH (results in 5M stock).

**Caution:** NaCNBH<sub>3</sub> is toxic and must be used in a fume hood.

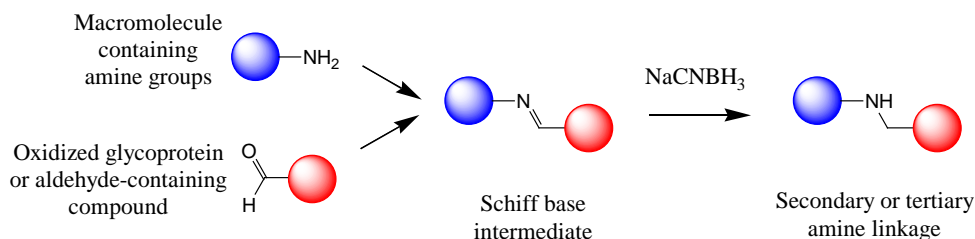
**Note:** Stock solution of NaCNBH<sub>3</sub> in NaOH is stable at room temperature or 4°C.

2. Dissolve an amine- or hydrazide-containing molecule (e.g., protein) at a concentration of 10mg/mL in PBS. If the protein is already in a solution containing Tris or other amine-containing buffer, dialyze or use a desalting column to exchange the sample into PBS (see Section B).
3. Mix the oxidized and desalted glycoprotein solution (prepared in Section A and B) with the protein solution in amounts necessary to obtain the desired molar ratio for conjugation. Often, the second molecule is reacted in 4- to 15-fold molar excess over the amount of oxidized glycoprotein.
4. Add 10µL of 5M AminoLink Reductant solution per milliliter of protein conjugation mixture (results in 50mM).
5. Allow reaction to proceed for 4-6 hours at room temperature.

**Note:** If more convenient, the reaction may be performed overnight at 4°C.

6. Block non-reacted aldehyde sites (quench the reaction) by adding of 50µL of Blocking Buffer per milliliter of conjugation solution and incubating the reaction for 30 minutes at room temperature.
7. Purify the conjugate from excess reactants using a desalting column (as described in Section B) or dialysis (see Related Thermo Scientific Products). Exchange the conjugate into a storage buffer suitable for the specific proteins.

## Additional Information



**Figure 1. Reaction scheme for reductive amination using Thermo Scientific AminoLink Reductant (sodium cyanoborohydride).**

## Related Thermo Scientific Products

20501	<b>AminoLink Plus Coupling Resin, 10mL</b>
44894	<b>AminoLink Plus Immobilization Kit</b>
21340	<b>EZ-Link™ Biotin-LC-Hydrazide, 50mg</b>
33015	<b>AMCA-Hydrazide, 5mg</b>
46185	<b>R-Phycoerythrin (PE), 2mg</b>
28372	<b>BupH™ Phosphate Buffered Saline Packs, 40 packs</b>
66382	<b>Slide-A-Lyzer™ Dialysis Cassette Kit</b>
89891	<b>Zeba™ Spin Desalting Columns, 5mL</b>
23260	<b>Glycoprotein Carbohydrate Estimation Kit</b>
20504	<b>Sodium meta-Periodate, 25g</b>

## Cited References

- Hermanson, G.T. (1996). *Bioconjugate Techniques*, Academic Press.
- Lane, C.F. (1975). Sodium cyanoborohydride—a highly selective reducing agent for organic functional groups. *Synthesis* 136-146.
- Dottavio-Martin, D. and Ravel, J.M. (1978). Radiolabeling of proteins by reductive alkylation with [14C]-formaldehyde and sodium cyanoborohydride. *Anal Biochem* **87**:562.
- Peng, L, Calton, G.J. and Burnett, J.W. (1987). Effect of borohydride reduction on antibodies. *Applied Biochem Biotech* **14**:91.

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