Carboxylation Kit With Citraconic Anhydride

Blocking of Free Primary Amines and adding Carboxylate Group

CITRACONIC ANHYDRIDE PROTEIN MODIFICATION KIT

(Cat. # 786-1651)

INTRODUCTION

G-Biosciences Carboxylation Kit With Citraconic Anhydride is designed for reversible blocking of primary amines so that directed conjugation or labeling can be performed. Citraconic anhydride reacts with primary amines and blocks them by creating an amide linkage and a terminal carboxylate. The linkage is stable at neutral to alkaline pH (pH >7) and at acidic conditions (pH 4) the amide linkage is rapidly hydrolyzed to release the citraconic acid and free the amines (Fig.1). This property makes citraconic anhydride a very useful tool for blocking free amines in proteins and other biomolecules.

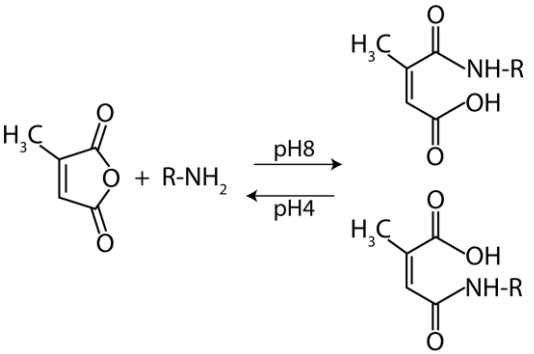


Fig.1: Amine group modification by citraconic anhydride

ITEM(S) SUPPLIED (Cat. # 786-1651)

Description	Size
Citraconic Anhydride	10 g
Optimizer Buffer™ VIII [5X]	2 x 25 ml

STORAGE CONDITIONS

The Kit is shipped at ambient temperature. Store at room temperature.

ADDITIONAL ITEMS REQUIRED

Tube-O-DIALYZER™ (Cat. #786-610 -786-624) or SpinOut™GT-600 (Cat. # 786-704) columns

PREPARATION BEFORE USE

Dilute Optimizer Buffer™ VIII [5X] with deionized water in ratio 1:4 (e.g. 1 ml of Optimizer Buffer™ VIII [5X] and 4 ml of deionized water) to get 1X Optimizer Buffer™ VIII solution.

PROTOCOL

Blocking primary amines on protein

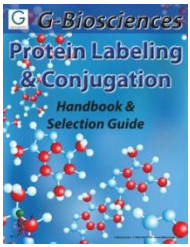
1. Dissolve the protein or other amine containing molecule in 1 X Optimizer Buffer™ VIII.
   NOTE: *For samples already in amine containing buffers perform a buffer exchange by gel filtration (SpinOut™GT-600) or dialysis (Tube-O-DIALYZER™) against 1 X Optimizer Buffer™ VIII.*
2. Add at least a 5-10 fold molar excess of citraconic anhydride to amines. Add the citraconic anhydride in small aliquots to maintain the solubility in the reaction mixture.
   NOTE*: Molecular weight of citraconic anhydride is 112.08.*
3. Incubate for 1-2 hours at room temperature, or 4°C overnight.
4. Remove any excess citraconic acid by gel filtration (SpinOut™GT-600*)* or dialysis (Tube-O-DIALYZER™).
5. To reverse the reaction, adjust the pH to ~4 by adding an appropriate acid.
6. Incubate for >3 hours to overnight at 30°C.
   NOTE: *As an alternative, treat with a final concentration of 1M hydroxylamine, pH10 for 3 hours at room temperature.*

REFERENCES

1. Dixon, H.B.F. and Perham, R.N. (1968) Reversible blocking of amino groups with citraconic anhydride. Biochem. J. 109:312-314

RELATED PRODUCTS

Download our Protein Labeling & Conjugation Handbook.



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

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