G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Optimizer-BlueBALLS™

For Establishing Optimal Detergent Concentration, Critical Micelle Concentration (CMC), & Optimal Extraction Of Membrane Proteins

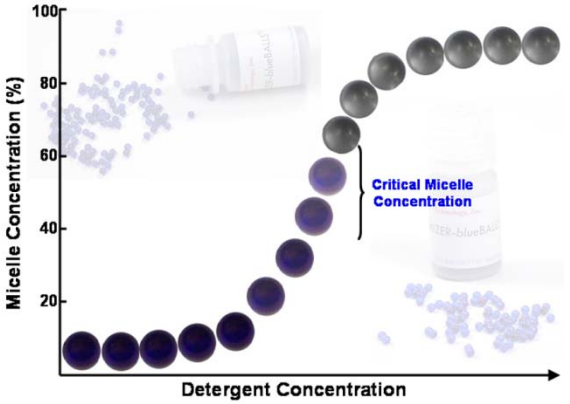
(Cat. # DGA01)

INTRODUCTION

The formation of micelles allows sequestering of hydrophobic molecules into the micelles. Hence, the CMC defines minimum concentration of detergent required to solubilize membrane proteins and other hydrophobic molecules.

The *CMC* of a detergent varies with temperature, pH, ionic strength, detergent concentration, purity and presence of organic agents in the detergent. Hence, the *CMC* values cited in literature may not be adequate for a given application. Using large excess concentration of detergent may pose problems during purification procedures. Measuring *CMC* is time consuming and requires use of expensive equipments not available in all laboratories such as surface tension and light scattering.

Brigitte Vulliez-Le Normand and Jean-Luc Eisele (*Analytical Biochem*. 208, 241-243. 1993) have described a simple hydrophobic dye solubilization method for determination of *CMC.* Solubilization of dye in a detergent solution occurs only if micelles are formed. The quantity of dye in detergent solution is directly proportional to the number of micelles. This method measures solubilization of dye in detergent solution. The *CMC* is determined by plotting optical density of solubilized dye against detergent concentration.



The point of inflection on the plot of observed data vs. detergent concentration corresponds to the *CMC* of a typical detergent. This method is simple and comparable to *CMC* determined by light scattering or surface tension methods. Furthermore, this method is applicable to all detergents, including steroid based detergents such as CHAPS, deoxycholate, cholate as well as non-steroid detergents like *beta*-octylglucoside.

The Optimizer-blueBALLS™ are simple to use hydrophobic blue dye coated balls. Just add the Optimizer-blueBALLS™ in extraction buffer and observe progress of dye extraction. The test and the progress of extraction can be easily observed with the naked eye. The test allows visual feedback for optimal time for extraction procedures.

ITEM(S) SUPPLIED (Cat. # DGA01)

Description	Size
Optimizer-blueBALLS™	500

STORAGE CONDITIONS

Shipped at ambient temperature. Store at room temperature.
Optimizer-blueBALLS™ is good for five years when stored and used properly.

ADDITIONAL ITEMS REQUIRED

Tubes, extraction buffers, and titer reading plates.

PROTOCOL

***NOTE**: Please note dye solubilization time depends on the nature of detergent, temperature, pH, ionic strength, detergent concentration, purity and presence of organic agents in the detergent buffer preparation. Dye solubilization time (or this test) may vary from a few minutes to 2-15 hours.*

A. Determination of Critical Micelle Concentration (CMC) of a Detergent

The CMC is determined by plotting optical density of solubilized dye against detergent concentration. The point of inflection on the plot of observed data vs. detergent concentration corresponds to the CMC of a typical detergent.

1. In a series of tubes transfer 0.25ml detergent solution (containing increasing concentration of the detergent to be tested for CMC).
2. Add 1-3 Optimizer-blueBALLS™ into each tube. Close the tube and incubate for 2-16 hours. Periodically vortex the tube.
   ***NOTE**: Shorter incubation time may be considered if dye solubilization is observed.*
3. At the end of incubation period, vortex the tube and centrifuge the tube at 5-6,000xg for 5 minutes. Transfer the extract to a titer plate and read the optical density at 630-650nm. Alternatively, examine the color of the extract with naked eye.
4. The *CMC* is determined by plotting optical density of solubilized dye against detergent concentration. The point of inflection on the plot of observed data vs. detergent concentration corresponds to the *CMC* of a typical detergent

B. Determination of "Optimal Extraction Time" for Extraction Buffers

1. Transfer 0.25ml extraction buffer into two 1.5 ml microfuge tubes.
2. Add 1-3 Optimizer-*blue*BALLS™ into <u>one</u> of the tube. Invert the tubes 2-3 times. Incubate the tube at room temperature. Mark the <u>second</u> tube "Control".
3. Make Periodic observation of the tubes, every 15 minutes - 1h.
4. Invert the tube 2-3 times and centrifuge the tubes 5-6,000xg for 5 minutes.
5. Examine the color of the test tube with naked eye and compare with the control tube. Alternatively, read the optical density of the extracts at 630-650 nm.

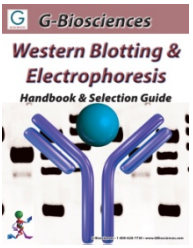
The beginning of solubilization of the dye into the extraction buffer is indicative of the solubilization of hydrophobic molecules or proteins. Increase in optical density with time is indicative of increasing amount of dye solubilized in the extraction buffer.

C. Determination of Optimal Extraction or Solubilization

Optimizer-blueBALLS™ may be directly added into the extraction protocol (or a test run extraction protocol). Release of blue dye may be monitored as an indication of satisfactory extraction or solubilization of membrane proteins.

RELATED PRODUCTS

Download our Western Blotting Handbook.



http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide/

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 7/25/2012 CMH

BIOSCIENCES®

www.GBiosciences.com