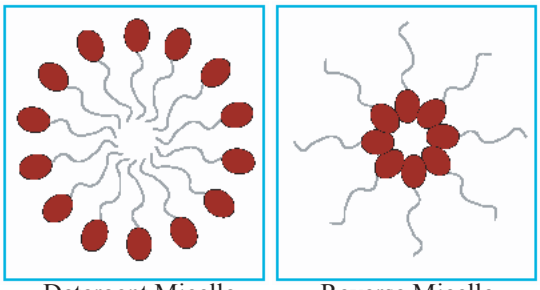
How To Use Detergents Effectively: The Optimizer blueBALLS™ Method

Detergents are a critical component of many lysis/ extraction buffers as they are required for the solubilization and extraction of hydrophobic proteins, however; simply adding detergent to an extraction buffer is not the best way to use detergents. The action of detergents is dependent on their property to form micelles, which are spherical arrangements of detergent molecules clustered so that their hydrophobic tails are buried inside the spheres and their hydrophilic head groups are on the surface. These micelles sequester the hydrophobic proteins allowing for their solubilization and extraction. By contrast, in hydrocarbon solvents, reverse micelles are formed where the hydrophilic portions are sequestered in the core and the hydrophobic portions form the outer shell.



Detergent Micelle (Aqueous medium) Reverse Micelle (Non aqueous medium)

An important factor in the usage of detergents is their Critical Micelle Concentration (CMC), which defines the minimum concentration of detergent at which molecules aggregate to form micellar structures that are required to solubilize membrane proteins and other hydrophobic molecules. Unfortunately for researchers, the CMC of a detergent varies with temperature, pH, ionic strength, detergent concentration, purity, and presence of organic agents in the detergent and therefore, the CMC value cited in literature may not be adequate for a given application. Often researchers use an excess of detergents to ensure complete solubilization and extraction of proteins and these excess concentrations of detergent may pose problems during subsequent purification procedures or other downstream applications. The ability to measure the CMC is often not available to many laboratories as the process requires expensive equipment, such as surface tension and light scattering, and is also time consuming.

G-Biosciences has developed a simple product and method to determine the optimal detergent concentration.

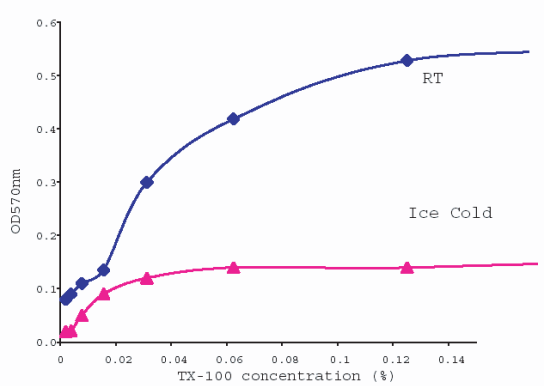
Optimizer *blue*BALLS™

Optimizer *blue*BALLS™ *are glass balls that have been coated with a hydrophobic blue dye that has the same properties as membrane proteins. Simply add* Optimizer *blue*BALLS™ *into your extraction protocol and then stand back and watch the extraction process as the blue dye is solubilized into the buffer. The solubilization of the dye only occurs when micelles have been formed in the solution, giving a simple visual guide to extraction.* Optimizer *blue*BALLS™ *are designed to work with all detergents, including the steroid-based detergents.*

THE OPTIMIZER METHOD

As stated above, one of the problems of using detergents is that excessive concentrations can lead to problems during subsequent purification and further downstream applications. To help the researcher overcome this limitation, G-Biosciences has designed this simple protocol to allow researchers to optimize their detergent concentrations.

This example experiment uses the commonly used Triton® X-100 detergent. Serial dilutions of Triton® X-100 were prepared in 25mM Tris-HCl pH 7.5 buffer and 0.2ml of each of these buffers were transferred to a 1.5ml microtube. One Optimizer *blue*BALL™ was added into each tube and incubated at room temperature or in an ice-cold bath for two hours, with periodical vortexing. After two hours, the tubes were centrifuged at 16,000*g* for 5 minutes. 0.15 ml supernatant from each tube was transferred to a titer plate and the absorbance was read at 570nm.



Detergent Optimization: A plot of released blue dye from a *blue*BALL™ in serial dilutions of Triton® X-100.

RESULTS & DISCUSSION

The optical density plot shows that the blue dye, behaving as a membrane protein or hydrophobic agent, did not dissolve when the Triton® X-100 concentrations were lower than the CMC, however the blue dye quickly dissolved when the concentration of Triton® X-100 reached a critical concentration, the CMC, indicated by an arrow at the point of inflection on the plot. This point of inflection on the plot of observed data vs. detergent concentration corresponds to the CMC of a typical detergent. Interestingly, in ice-cold conditions, solubilization of the hydrophobic blue dye was very limited, even after prolonged incubation. At room temperature, solubilization of the dye occurred at a faster rate, approximately 3- to 5-times faster. The results demonstrate that the concentration of detergent, as well as the extraction temperature, had strong influence on the effectiveness of the extraction buffer. Use of Optimizer *blue*BALLS™ provides a simple method for optimization of extraction conditions and avoids the use of excessive concentration of detergents, as the release of blue color is indicative of solubilization.

ORDERING INFORMATION

Cat. #	Desription/Size	US Price
DGA01	Optimizer *blue*BALLS™/ 500	$108.00

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