

Tips and tricks of the trade

Bradford goes Hellenic

Challenging common belief is one of the main driving forces in research. One such example is given by Christos Georgiou and his colleagues at the University of Patras, Greece, who wondered whether the previously proposed mechanisms of the Bradford assay makes any sense.

Lab Hint

Blue is the archetypical 'Greek' colour. Just think of the bright blue Greek sky, the spectacular blue of the Greek sea, the blue painted shutters in the typical Greek villages and, not to forget, the blue stripes in the Greek flag. This omnipresence of blue colour in Greece may have inspired Christos Georgiou, Konstantinos Grintzalis and Ioannis Papapostolou from the University of Patras and George Zervoudakis from the Technological Institute of Mesologgi, to take a closer look at the mechanism of Coomassie Brilliant Blue G-250 (CBB) binding to proteins and develop a very sensitive new CBB protein assay (*Anal. Bioanal. Chem.* 391:391-403, 2008).

Though almost every technician or life science researcher around the globe uses, or at least knows of the CBB dye binding or Bradford assay to quickly determine protein concentrations, the underlying mechanism of CBB-protein binding is still a matter of debate. CBB is a triphenylmethane dye, originally developed for the textile industry, that exists in three ionic species depending on the pH value: doubly protonated, i.e. cationic, at pH < 0.3 (red colour), neutral at a pH around 1.3 (green) and unprotonated, i.e. anionic, at pH > 1.3 (blue).

So the question is: which species actually binds to proteins in the Bradford assay? Since experiments have shown that the cationic species does not bind proteins at all, it has been speculated that anionic CBB forms a complex with proteins leading to a measurable increase in absorbance. As the Bradford assay is usually done at a pH below 1.3, however, Georgiou and his colleagues won-

dered where the anionic CBB species should come from. They reassessed the absorbance increase at pH 0.64 (at which the Bradford assay is performed) and came to the conclusion that the enhancement of absorbance must be caused by binding of the neutral, and not the anionic species, to proteins. According to the Greek group, the main binding forces are hydrophobic interactions of neutral CBB with hydrophobic amino acids (Trp, Phe and Tyr) and electrostatic interactions between basic amino acids and sulfonic groups of CBB.

Based on their findings, Georgiou *et al.* readjusted the settings of the Bradford assay and developed an improved hydrophobic version that may detect nanogramme (ng) quantities of protein.

Here is what you need for the Greek CBB assay in standard and microplate format (you may find a slightly different protocol for a microassay in the original paper). At first, one requires a CBB-HCl stock solution that is prepared by dissolving 60 milligrammes (mg) CBB in 100 millilitres (ml) 1N HCl. The solution is stirred for 40 minutes and filtered through a Whatman no.1 filter and stored at 4 °C.

According to the authors, the solution is stable for months when protected from light. To prepare 20 ml hydrophobic assay reagent (CBB-TCA reagent), absolute ethanol and trichloroacetic acid are added to the stock solution to give final concentrations of 1% and 2%, respectively. Subsequently, the pH is adjusted to 0.4 with solid Na₃PO₄·H₂O and the reagent is cleared from the blue particulate formed



Greece coast views are a (blue) feast for the eyes.



Developed a highly sensitive Coomassie Brilliant Blue protein binding assay: Christos D. Georgiou.

by centrifugation at 5,000 g for five minutes at room temperature.

The assay itself is a similar no-brainer like the classic Bradford assay. Just mix 0.05 ml of protein solution with 0.95 ml CBB-TCA reagent, incubate 5 to 10 minutes at room temperature and measure the absorbance at 610 nanometres (nm). The mandatory standard curve is established by applying a solution of bovine serum albumin with a concentration of 2 to 60 microgrammes (µg) per ml; standard solutions made of lysozyme, cytochrome c, haemoglobin or pepsin may work as well.

The most remarkable feature of the Hellenic Bradford assay, however, is its high sensitivity. The standard and microplate versions may detect as little as 100 ng and 50 ng of protein, respectively. These are pretty low amounts. Just compare the sensitivity of the Greek standard assay with that of commercially available Bradford assays. Even the most sensitive Bradford assay versions from the companies Sigma and Pierce are 100- and 40-fold less sensitive than the Hellenic standard assay. Not to mention that the latter is supposed to be much cheaper than the commercial assays.

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Do you have any useful tips?

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