Sample Quantity Estimation

Estimating Protein Quantity

An excellent method of estimating the quantity of protein is based on density in a 1x8mm band on an SDS-PAGE gel.

A sharp 1 x 8 mm band on a gel (0.75mm) holds on average 1 μg when saturated and will be a very dark Coomasie Blue stain. Be conservative and avoid convincing yourself that volume of gel contains more. You scale your estimate relative to this by both intensity *and area (volume).*

1. Evaluate your intensity on a scale of 1 to 5 where 5 is the darkest Coomassie band you observed in your career, 1 is a very faint, near threshold Coomassie, and 3 is an average stain.

2. Estimate the gel area in mm^2

3. Calculate the micrograms of protein as follows

$$\mu g\ protein = (intensity / 5) \times (area / 8)$$

4. Once you have an estimation of mass and molecular weight, calculate the picomoles of protein present

$$picomole\ protein = 1000 \times \mu g\ protein / M.W.\ (in\ kDa)$$

Thus, if you have a *very* darkly stained 1 x 8 mm band (~1ug), for a 25 kDa protein, you have 40pmol present . You would, however, have only 10pmol for a 100 kDa protein, and only 4pmol for a 250 kDa protein.

Do not rely on comparison to MW markers as a method since by doing so you are normalizing back to what was present in the tube, *not what is available in the gel for digestion.*