




## Modul 5 (alternative): Rapid Coomassie Assay

The rapid Coomassie assay is a very simple and fast semi-quantitative protein detection test. Coomassie is a blue dye that binds to basic amino acids (like e.g. arginine) and serves as an indicator for proteins. Of course, staining intensity depends on the number of basic amino acids in a given protein. The amount of a certain protein will be underestimated if it has no or only a few basic amino acids but in a protein mixture, you will get a sufficiently good estimate.

-  *Read the protocol completely before beginning with the experiment*
-  *Which result do you expect with the fractions of the affinity purification? Draw the expected staining results with a blue marker into the circles in Fig. 1!*

### Step 1: Preparations

-  Cut a stripe of 2 to 3cm width and 10cm length from the Whatman paper. Draw four circles on the paper as shown in Fig. 1 and make sure that approx. 2cm are left free at one end for handling. Label the circles with the abbreviations of your samples (L, W3, E1, E2). (H<sub>2</sub>O is the negative control.)

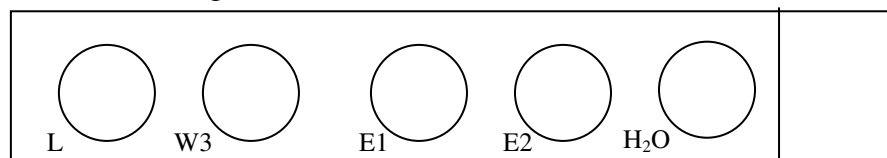





Fig. 1: Test stripe for rapid Coomassie assay

### Step 2: Loading the samples

-  Pipette 5  $\mu$ L of the respective samples in the circles on the Whatman paper. Try to have all dots of equal size.  
Dry the Whatman paper either on the bench or on a heating block (40-50 °C).

### Step 3: Staining reaction

-  When the Whatman paper is completely dry, hold it on the free end and dip it into Coomassie solution for a few seconds. Immediately after that, dip it into a beaker of water to remove excess dye. You may then rinse it under running water for a few seconds. Dry the stripe on the bench or on a heating block and evaluate the results.

-  *Paste the stripe into the field below and compare the results with your expectations above. Give a brief explanation of the results.*