determination of proteins from sediment samples

Principle:

The proteins are extracted from the cells and divided by NaOH and temperature into reactive fragments. The temperature may not be higher than 60°C, since otherwise the fragments become too small and are not detectable any longer. Measurements are done photometrically (colour reaction of the protein fragments with Coomassie blue).

Accessories:

Photometer
Plastic cuvettes (half micro)

Execution:

Immediately after sampling the sediment (5ml syringe) is shock-frozen with -80°C, then with -18°C stored. To the first hydrolysis the sediment is cut in centimetre horizons and filled in numbered test tubes.

Now the sediment is dried with 60°C at least for 48h (it must be completely drying).

Then 3ml 0,5N NaOH are admitted, tubes are whirled and finally hydrolyzed for 2 hours in the water bath with 60°C.

Afterwards, once again whirled and centrifuged with 3500 U/min for 10 minutes. The supernatant (sample) is decanted and the sediment is washed with 2ml 0,5N NaOH (whirling and 10 minutes with 3500 U/min. to centrifuge).

Both extracts are combined and measured (1st hydrolysis).

If further extractions should be desired, the sediment is dried again etc..

The individual hydrolyses run off as described above. After the 6th hydrolysis all excerpts are combined, the volume is again determined and the protein content is measured.

Measurement:

Preparation:

Test tubes (some more than necessary for the measurements) are filled with 2 ml Coomassie blue (keep cool as for a long time as possible to); prepare plastic cuvettes (half micro), stop clock and Whirlmix. The thawed out hydrolysate may not stand over longer time, since then a concentration-reduction takes place. One should try only as much to thaw out as also in the next hours is measured.

Measuring procedure:
One measures with a wavelength of 595nm and with a gap width of 2.

At first the cuvettes blank values are determined using 0,5N NaOH (subtract afterwards from the sample values or use auto-zero).

Pipette 0,35ml hydrolysate into a test tube already filled up with coomassie blue, whirl for 10 seconds. The mixture is poured into the appropriate cuvette and measured after 2 minutes. During this response time further mixtures can be prepared. Per sample always two mixtures are prepared and measured! If the deviation is larger than 20 fluorescence units, still another further parallel must be made. The response time must be kept absolutely exactly (stop clock).

After the measurement the cuvettes are soaked immediately in soap sud, so that the dyestuff can be well removed (rinse cuvettes carefully, otherwise a blue pass remains, and the blank value is much too high and can't be adjusted any longer). The cuvettes can be used well rinsed up to five times.

Since the dyestuff is instable, one calibration must be measured per measuring day. The calibration solutions can be kept frozen. Repeated thawing out and freezing harms! Therefore divide the 100ml master solution in 10ml portions and freeze only in such a way. The calibration curve is linear between 0 and 150µg Globulin/ml. If the protein concentration of the samples exceeds this range, mixture must be diluted.

Computation:

µg -Gl.Äqu./5cm3 Sed. after 6 hydrolyses = 100%
µg -Gl.Äqu./5cm3 Sed. after 1 hydrolysis = x%
x% = relative protein concentration of the first hydrolysis

- multiply by the dilution, with the first hydrolysis x6,13, with six hydrolyses x 6x6,13
- divide by the sediment volume :1,13cm3
- convert with water content, because 1ml is not equal with 1cm3

Reagents:

0,5N NaOH:
20 g NaOH is solved in a litre of Aqua bidest..

Coomassie blue:
40 mg Serva blue are solved in approx. a half litre of Aqua bidest.. In addition comes 100ml phosphoric acid and 50 ml ethanol. Fill up with Aqua bidest. to 1 litre. The final solution must be whirled before use at least 24 hrs..

Calibration master solution:
10mg y-Globulin are solved in 50ml 0,5N NaOH.
y-Globulin as powder has to be stored with +4°C, as master solution with -18?C.

Standards:

Protein conc. [µg y-Globulin/ml]	Master solution [µl]	0,5N NaOH [µl]
0	0	3000
4	50	2950
10	150	2850
20	300	2700
30	450	2550
40	600	2400
50	750	2250
70	1050	1950
100	1500	1500

Chemical list:

NaOH(Merck No. 6498)
Serva blue (Serva No. 35050)
y-Globulin (Serva No. 22550)
85% ortho phosphoric acid (Merck No. 573)
ethanol, p.A. (Merck No. 983)