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卵白素（鸡蛋）

UNIT DEFINITION：

The amount of protein that will bind one microgram of d-biotin at pH 8.9.

ASSAY METHOD：

Avidin is assayed by determination of its biotin-binding capacity spectrophotometrically by the method of Green .

REAGENTS：

1. 0.2 M Ammonium carbonate buffer, pH 8.9.

2. 0.001 M d-Biotin, (0.244 mg/ml) in ammonium carbonate buffer, pH 8.9.

3. Avidin solution (0.2 mg/ml) in ammonium carbonate buffer, pH 8.9. Prepare fresh.

PROCEDURE：

1. Set spectrophotometer at 233 nm.

2. Pipette 3 ml of avidin into blank and sample quartz cuvettes. Record absorbance at 233 nm.

3. Add 10 µl of d-Biotin to sample cuvette and record change in absorbance at 233 nm.

4. Continue adding 10 µl biotin at a time to the sample cuvette until no further increase in absorbance at 233 nm occurs after three successive biotin additions.

5. Plot the number of aliquots of biotin used versus absorbance at 233 nm. Determine the equivalence point by drawing a straight line through the linear absorbance portion of the curve and by drawing a line through the portion where no further increase in absorbance at 233 nm occurs. The intersection of these two lines is

the equivalence point. By extrapolation, determine the number of aliquots of biotin represented by the equivalence point.

CALCULATION：

$$\text{Activity (U/mg)} = \frac{(\#\text{ aliq. at eq. pt.})(\text{vol. biotin aliq. in ml})(\text{biotin conc. µlg/ml})}{(\text{mg avidin in 3 ml})}$$