

09753 Albumine Fluorescence Assay Kit ¹⁾

Introduction

The specific and sensitive determination of albumin in biological fluids is required in many areas of biomedical sciences ²⁾. Assays suitable for the determination of low concentrations (<100 mg/l) of albumin in natural matrices are either nonspecific for albumin and rather test total protein content (dye binding methods) or use complicated and costly procedures (immunoassays).

The new dye albumin blue 580 (AB 580) ³⁾ made accessible now an easy, robust, sensitive and specific assay for albumin [5].

Kit content

– Reagent A:	solution of albumin blue 580 in 2-propanol	10 ml	Fluka 05497
– Reagent B:	buffer solution pH 7.0 +/- 0.2	2 x 250 ml	Fluka 79438
– Calibrator albumins:	albumin from human serum (HSA)	2 x 1 g	Fluka 05418
	albumin from bovine serum (BSA)	10 g	Fluka 05476
– Calibrator diluent	buffer solution pH 6.0 +/- 0.5	100 ml	Fluka 09761
– Instruction manual			

Working solutions

Assay reagent	- mix 2.0 ml reagent A with 100 ml reagent B
	- absorbance A should be 0.18 +/- 0.02 (582 nm, 1 cm-cuvettes)
	- store in glass bottle and prepare fresh each day
Calibrator solutions	- prepare an albumin stock solution of 2000 mg/l with dist. water (this stock solution is stable for at least 1 week when kept at 0-4 °)
	- dilute with calibrator diluent to final concentrations of 2.0, 10, 30, 100, 200 mg/l, respectively

Instrumentation, settings

Spectrofluorometer	bandpasses (exc. and em.) 3 nm, λ_{ex} 600 nm ⁴⁾ , λ_{em} 630 nm ⁵⁾
Cuvette	1 cm standard fluorescence cuvette
Temperature	room temperature

Sample preparation

Samples should be freed of unsolved particles (e.g. by centrifugation). Further preparation is often not necessary as shown in [4].

¹⁾ This assay was developed and described by O.S. Wolfbeis and coworkers (see references below)

²⁾ For a short summary of the diagnostic importance of albumin excretion rate measurements in urine see references [2] and [4]; for comprehensive overviews see lit. 1.-21. in [4]

³⁾ Earlier named „Albumin Blue 633“ corresponding to the wavelength of the HeNe-laser, by which it has been excited [1].

⁴⁾ $\lambda_{max,abs.}$ (unbound AB580) ~580 nm

⁵⁾ slightly offpeak

