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Product Information

09753
Albumine Fluorescent Assay Kit [1)]

Product Description:
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	Sigma-Aldrich 05497
– Reagent B:	buffer solution pH 7.0 +/- 0.2	2 x 250 ml	Sigma 79438
– Calibrator albumins:	albumin from human serum (HSA)	2 x 1 g	Sigma A3782
	albumin from bovine serum (BSA)	10 g	Sigma A2153
– Calibrator diluent	buffer solution pH 6.0 +/- 0.5	100 ml	Sigma-Aldrich 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 solution should be stable for at least 1 week when kept at 0-4 C. Dilute with calibrator diluents 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)]. Use a 1 cm standard fluorescence cuvette and 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].

Assay procedure
Mix 0.5 ml of sample/calibrator solution with 2.5 ml assay reagent. Measure fluorescence immediately.

Calibration
The calibration curve approximates well to

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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 exited [1].
4) $\lambda_{max.,abs.}$(unbound AB580) ~580 nm
5) slightly offpeak

$$y = \frac{Ax}{(1 + Bx)} + C$$

y = relative fluorescence emission intensity
x = albumin concentration [mg/l]
A, B, C = fit parameters

albumin	A	B	C
human serum (HSA)	2.15	0.006	4.77
bovine serum (BSA)	0.81	0.0016	5.21

(table 1; for parameters of further albumin species see [5])

Assay scope and limitations

Limit of detection (LOD)	~0.4 mg/l
Upper limit	~200 mg/l
Interference by proteins other than albumines	less than 1% response with same amounts of protein
Interferences by additives	certain detergents and organic solvents disturb
Sample properties	pH ideally between 6 and 8 ionic strength > 200 mmol/l sample with extrem pH, ionic strength and buffer capacity should be diluted

Intra-assay precision

HSA calibrators [mg/l]	coefficients of variation
1.0	3.6 %
10	1.2 %
30	1.1 %
100	0.6 %
200	0.6 %

Note: cuvette solution should be measured within 5 min. of preparation

Kit storage

The kit should be stored at 0-4°C

1. Byrdwell, W. C, and Neff, W. E., Dual parallel electrospray ionization and atmospheric pressure chemical ionization mass spectrometry (MS), MS/MS and MS/MS/MS for the analysis of triacylglycerols and triacylglycerol oxidation products. Rapid Commun. Mass Spectrom., 16(4), 300-319 (2002).
2. Stoll, T., et al., High-performance liquid chromatographic separation and on-line mass spectrometric detection of saturated and unsaturated oligogalacturonic acids. Carbohydr. Res., 337(24), 2481-2486 (2002).
3. Brunner, N. A., et al., Crystallization and preliminary X-ray diffraction analysis of the NADdependent non-phosphorylating GAPDH of the hyperthermophilic archaeon Thermoproteus tenax. Acta Crystallogr. D Biol. Crystallogr., 56(Pt 1), 89-91 (2000).
4. B.A. Hodson, et al., J. Chromatogr., 565 (1991).

Precautions and Disclaimer:
For Laboratory Use Only. Not for drug, household or other uses.