Albumin Blue Fluorescent Assay Kit

(version C2)

Catalog No. 15002

TABLE OF CONTENTS

Introduction

Active Motif's Albumin Blue Fluorescent Assay Kit simplifies albumin quantification by providing highly sensitive detection reagents in a convenient and easy to use format. Currently assays that are suitable for the determination of low concentrations (<100 mg/L) of albumin in natural matrices are either nonspecific for albumin or test total protein content such as in dye binding methods. In addition, these methods are also complicated and costly. In contrast, the Albumin Blue Fluorescent Assay Kit is sensitive, specific and easy to use.

The kit provides lyophilized dye reagent, buffers and HSA for preparation of standards. Simply resuspend the lyophilized dye in isopropanol to create the concentrated stock solution, dilute the stock solution, load 150 µl into the wells of a microplate, add 25 µl of sample, mix, then read the fluorescence. The intensity of the fluorescent signal is directly proportional to the albumin concentration in the sample.[1-4] The kit is suitable for use with both individual cuvettes and high-throughput 96 well plates.

Advantages

- Quick and Easy to Use
- Unmatched precision
- Sensitive - detection limit (~0.5 mg/L in urine and serum samples)
- Highly selective - no interference from other proteins or lipids
- High-throughput compatible

product	format	catalog no.
Albumin Blue Fluorescent Assay Kit	250 reactions*	15002

*Sufficient components are provided for performing 250 assays using fluorescent-based detection. This assay can also be easily adapted for use in smaller or larger formats such as 384-well plates or cuvettes.

Kit Components and Storage

The Albumin Blue Fluorescent Assay Kit is for research use only. Not for use in diagnostic procedures. Kit components arrive at room temperature. We recommend storing each component at the temperatures recommended in the table below:

Reagents	Quantity	Storage
Dye Reagent AM3	250 reactions	4°C for 6 months
Buffer A	50 ml	4°C for 6 month
Buffer B	10 ml	4°C for 6 months
HSA	0.2 mg	4°C for 6 months

Additional Materials Required

P200 and Multi-channel pipettor
Multi-channel pipettor reservoirs
Fluorescent detector
Black microtiter plates or cuvettes
Isopropanol (for reconstituting the Dye Reagent)

Preparation of Reagents

Dye Reagent Stock Solution

The Dye Reagent is supplied lyophilized. Proper reconstitution of the dye is pivotal to the performance of the assay. Prepare the Dye Reagent Stock Solution by resuspending the lyophilized Dye Reagent in 1 ml isopropanol in the provided amber vial. We recommend using an end-over-end rotator at room temperature to mix the dye for 30 minutes to ensure complete reconstitution. Once reconstituted the solution can be stored at 4°C for 6 months.

Dye Reagent Working Solution

Prepare the Dye Reagent Working Solution by diluting the Dye Reagent Stock Solution 1:50 with Buffer A. This solution should be prepared fresh on the day the assay is performed.

Reagent.	10 rxns	50 rxns	100 rxn
Dye Reagent Stock Soln	33 µl	158 µl	315 µl
Buffer A	1.617 ml	7.742 ml	15.435 ml

Stock HSA Solution

The HSA is supplied lyophilized. Prepare the Stock HSA Solution by resuspending the lyophilized HSA in 1 ml of Buffer B in the provided tube to make a 0.2 mg/ml solution. This stock solution can be stored at -20°C for 6 months.

Protein Quantification Kit Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

1. Remove kit contents from 4°C and bring all components to room temperature before use.
2. Set up a HSA Standard Curve in duplicate using the following concentrations: 200, 100, 50, 25, 12.5, 6.3, 3.2 and 0.0 µg/ml. (See table below for suggested layout).
   Note: We recommend you use a P200 single-channel pipet to set up the standard curve.
3. Add 50 µl of 200 µg/ml HSA in Buffer B to wells A1 and A2.
4. Add 25 µl of Buffer B to wells B1 through H1 and B2 through H2
5. Transfer 25 µl HSA solution (200 µg/ml) from wells A1 and A2 into wells B1 and B2.
6. Mix wells B1 and B2 by pipetting.
7. Transfer 25 µl from well B1 to C1 and B2 to C2.
8. Mix wells C1 and C2 by pipetting.
9. Transfer 25 µl from well C1 to D1 and C2 to D2.
10. Continue this procedure to wells G1 and G2. After mixing, discard 25 µl of solution from wells G1 and G2.
11. Wells H1 and H2 are blanks and should contain only 25 µl of Buffer B.

	1	2	3	4	5	6	7	8	9	10	11	12
A	200 µg/ml	200 µg/ml	–	–	–	–	–	–	–	–	–	–
B	100 µg/ml	100 µg/ml	–	–	–	–	–	–	–	–	–	–
C	50 µg/ml	50 µg/ml	–	–	–	–	–	–	–	–	–	–
D	25 µg/ml	25 µg/ml	–	–	–	–	–	–	–	–	–	–
E	12.5 µg/ml	12.5 µg/ml	–	–	–	–	–	–	–	–	–	–
F	6.3 µg/ml	6.3 µg/ml	–	–	–	–	–	–	–	–	–	–
G	3.2 µg/ml	3.2 µg/ml	–	–	–	–	–	–	–	–	–	–
H	Blank	Blank										

12. **Sample wells:** For albumin determination of unknown samples, prepare a series of dilutions with Buffer B, for example: 1:50, 1:100 and 1:200. Pipette 25 µl into each well. Duplicates of each sample are recommended.
NOTE: A clear urine sample is recommended for this assay. If the sample is turbid then it should be cleared by a centrifugation step.

13. Add 150 µl/well of **Dye Reagent Working Solution** (see Preparation of Reagents section on page 2 for directions on preparing the Dye Reagent Working Solution) and mix by pipetting up and down.

14. Incubate for 5 minutes at room temperature (20-25°C) with gentle shaking.

15. Measure the fluorescence (excitation: 560 nm, emission: 620 nm). Best results are obtained if samples are read within 5 minutes of preparation, however, the signal intensity is stable for up to 30 minutes. (**NOTE:** When measuring fluorescence, ensure that the gain settings are set to optimal (~ 120-140 gain), the number of flashes are set to 3 with no lag time and that the integration time is ~ 40 µs.

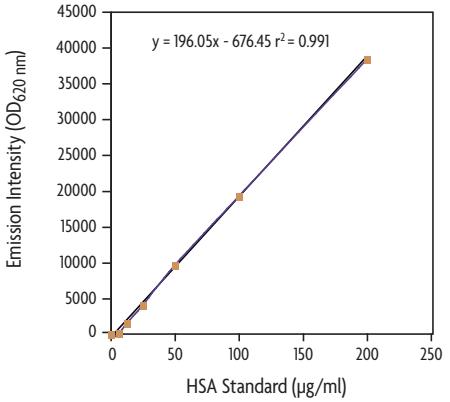
16. Use the standard curve to calculate the albumin concentrations of the unknown samples. For the HSA standard curve, fit with the function y= Ax + B. R^2 should be over 0.95.

Calculation of albumin concentration using the HSA standard curve

Average the duplicate readings for each standard and sample. Then subtract the values obtained from the zero standard. Plot the fluorescence for the standards against the quantity of the standards and draw the best fit curve. To quantify the amount of albumin in the samples, find the fluorescence value for the samples on the y-axis and extend a horizontal line to the standard curve. At the intersection point extend a vertical line to the x-axis and read the corresponding standard value. Note: If the samples have been diluted, the value read from the standard curve must be multiplied by the dilution factor. Alternatively, the equation that best fits the curve can be used to determine the sample concentration.

Example curve:

The following standard curve is provided for demonstration only. A standard curve should be made every time an experiment is performed.



References

1. Kessler M.A. and Wolfbeis O.S. (1992) *Anal. Biochem*. 200: 254-259.
2. Kessler M.A. *et al* (1992) *Clin. Chem*. 38(10): 2089-2092.
3. Kessler M.A. *et al* (1997) *Clin. Chem*. 43(6): 996-1002.
4. Kessler M.A. *et al* (1997) *Anal. Biochem.* 248: 180-182.

Appendix

Section A. Troubleshooting

I. Interference by other proteins

Several other proteins were tested for interference at a concentration of 2000 mg/l. Since the fractions of the proteins tested were not essentially free of albumin, part of the response may have originated from albumin. This implies an even lower interference by these proteins. A number of common contaminants have been tested with Albumin Blue Fluorescent Assay, and most are well tolerated; however, samples containing high concentrations of free amines are not recommended.

Contaminant	Response (%) (human albumin = 100%)
Human transferrin	0.5
Bence Jones protein	0.1
Lactoglobulin	0.8
Lysozyme	0.2
Pepsin	0.6
Trypsin	0.2
Bovine γ-globulin	0.2

II. Excitation and Emission Filters

We recommend using filters with the following ranges: 540-580 nm Excitation and 600-640 nm Emission.

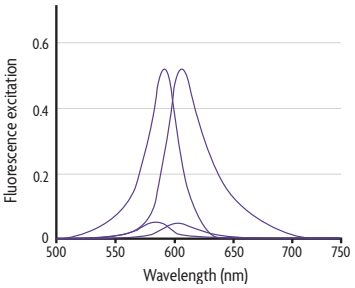


Figure 1: Absorption/emission spectra of free/bound dye.
Normalized absorption and emission spectra of free (lower curves) and conjugated dye (upper curves) in the Albumin Blue Assay.

III. Assay limits and recommendations

A clear urine sample is recommended for this assay. If the sample is turbid then it should be cleared by a centrifugation step. Although the assay is relatively robust with minor sensitivities to pH between 6 and 8 and ionic strength above 200 mmol/L, potential sources of error need to be addressed. Thus samples with extreme pH, ionic strength or high buffer capacity should be diluted to warrant constant assay conditions. Evaporation of isopropanol from the microtiter plate well and temperature changes must be avoided. Best results are obtained if samples are read within 5 minutes of preparation.

Section 4: Technical Services

If you need assistance at any time, please call or send an email to Active Motif Technical Service at one of the locations listed below.

Active Motif North America

Toll free: 877.222.9543
Direct: 760.431.1263
Fax: 760.431.1351
E-mail: tech_service@activemotif.com

Active Motif Europe

Direct: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan

Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China

Direct: (86)-21-20926090
Cell Phone: 18521362870
E-mail: techchina@activemotif.com

Visit Active Motif online at **activemotif.com**