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Working

UC Davis - Uninary Albumin Excretion (UAE) Protocol

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ABSTRACT

Summary:

Albumin blue dye is a stain for the specific and sensitive spectrofluorometric determination of albumin in natural matrices. AB 580 binds to the albumin present in urine samples and the fluorescence can be quantified using a fluorimeter.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=98&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Albumin	A6414	Sigma Aldrich
Calibrator Diluent	09761	Sigma Aldrich
Albumin Blue 580 Potassium Salt Solution	05497	Sigma Aldrich
Buffer	79438	Sigma Aldrich
Microplate (for fluorescence)		
Fluorimeter		

MATERIALS TEXT

Reagent Preparation:

Standards – Dilute 10 mg of albumin with 5 ml of Calibrator Diluent to make a 2000 mg/ml stock. Then dilute the stock 1:9 by adding 20 µl of stock to 180 µl of Calibrator Diluent to make a 200 mg/ml standard. Dilute the 200 mg/ml standard 1:1 with Calibrator Diluent to make 100,50,25,12.5,6.25 standards.

Calibrator Diluent – ready to use

Working reagent – Mix 2 ml of Albumin Blue 580 Potassium Salt Solution with 100 ml of Buffer.

Note:
Sigma-Aldrich RRID:SCR_008988

1 Prepare working reagent.

Prepare standards by serially diluting 200 mg/l standard 1:1 to make 100, 50, 25, 12.5, 6.25 standards.

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3 Add 25 µl of standard and sample to each well.

4 Add 125 µl of working reagent. Read in fluorimeter using 590 nm excitation and 616 nm emission.

IMPORTANT: *Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.*

5 Use a polynomial 2nd order curve fit to construct a standard curve. Interpolate the values of the unknowns using the standard curve.