MAY 15, 2019

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.yw5fxg6

External link:

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

Protocol Citation: Peter Havel 2019. UC Davis - Uninary Albumin Excretion (UAE) Protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yw5fxg6>

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Protocol status: Working
We use this protocol and it's working

Created: Mar 07, 2019

Last Modified: May 15, 2019

PROTOCOL integer ID:
21181

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.

Keywords: Albumin blue dye, Uninary Albumin Excretion (UAE)

MATERIALS

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Albumin Sigma Aldrich Catalog #A6414

Calibrator Diluent Sigma Aldrich Catalog #09761

Albumin Blue 580 Potassium Salt Solution Sigma Aldrich Catalog #05497

Buffer Sigma Aldrich Catalog #79438

Microplate (for fluorescence) Contributed by users

Fluorimeter Contributed by users

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](https://www.sigmaaldrich.com/US/en/product/008988)

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