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## UC Davis - Uninary Albumin Excretion (UAE) Protocol

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**Protocol status:** Working

**We use this protocol and it's working**

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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.



## Materials

### MATERIALS

⊗ Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A6414**

⊗ Calibrator Diluent **Merck MilliporeSigma (Sigma-Aldrich) Catalog #09761**

⊗ Albumin Blue 580 Potassium Salt Solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #05497**

⊗ Buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #79438**

⊗ Microplate (for fluorescence)

⊗ Fluorimeter

### 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.
- 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 2<sup>nd</sup> order curve fit to construct a standard curve. Interpolate the values of the unknowns using the standard curve.