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Working

Yale - Blood and Urine Creatinine [↗](#)

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ABSTRACT

Summary:

Procedure followed to detect the concentration of creatinine in serum, plasma, and urine.

EXTERNAL LINK

<https://mmpc.org/shared/document.aspx?id=204&docType=Protocol>

MATERIALS

NAME	CATALOG #	VENDOR
Acetonitrile	9012-03	J.T. Baker
LC/MS/MS Buffer		
D3-Creatinine	W212P16	CDN Isotopes

MATERIALS TEXT

Reagent Preparation:

Reagent 1: Acetonitrile: As supplied by vendor

Reagent 2: LC/MS/MS Buffer: 85% water and 15% of 8.5mM Ammonium Acetate.

Reagent 3: D3-Creatinine standard: (5 mg/dL)

- 1 Fill 1.5mL eppendorf tubes with 80µL of acetonitrile.
- 2 Add sample:
 - a. Use 20µL of plasma or serum/tube
 - b. Use 6 µL of urine/tube
- 3 Add 6.5 µL of d3-creatinine (5mg/dl) to each tube.
- 4 Vortex each sample for 5 seconds.

Spin each sample at 4°C for 10 minutes

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6 Transfer supernatant to LC/MS/MS vials.

7 Dry samples in speed vacuum.

8 Resuspend pellet in 75µL of LC/MS/MS Buffer

9 LC/MS/MS (liquid-chromatography/ tandemmass spectrometry) analysis:

LC- Column: Isocratic using a Hamilton PRP-X200 column. Mass Spectroscopy: MRM mode with parent/daughter ion pairs of 114/44 for creatinine and 117/47 for d3-creatine. (**Ref:** *Takahashi N, Boysen G, Li F, Li Y, Swenberg JA. Tandem mass spectrometry measurements of creatinine in mouse plasma and urine for determining glomerular filtration rate. Kidney Int. 71: 266-271, 2006.*)



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