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WORKS FOR ME

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# Canine/feline serum or plasma deproteinization for amino acid analysis on Biochrom

DOI

[dx.doi.org/10.17504/protocols.io.877hzrn](https://dx.doi.org/10.17504/protocols.io.877hzrn)Amanda Blake<sup>1</sup>, Jan Suchodolski<sup>1</sup><sup>1</sup>Texas A&M University - College Station

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COMMENTS 0

## ABSTRACT

This protocol describes the deproteinization process that all canine and feline serum and plasma research samples undergo prior to amino acid analysis with a Biochrom 30+ Amino Acid Analyzer at the Gastrointestinal Lab, Texas A&M University. L-norleucine is used as an internal standard.

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## KEYWORDS

amino acids, deproteinization

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MATERIALS TEXT

## MATERIALS

☒ 750µL Nonsterile Micro-Centrifugal PVDF Membrane Filters, 0.2µm pore size **Thermo Fisher Catalog #F2517-5**

Step 5

☒ Amino acid standards physiological acidics and neutrals **Millipore Sigma Catalog #A6407**

☒ Amino acid standards physiological basics **Millipore Sigma Catalog #A6282**

☒ L-Norleucine **Millipore Sigma Catalog #N8513-100mg** Step 2

☒ 5-Sulfosalicylic acid dihydrate BioXtra >=99.0% **Sigma – Aldrich Catalog #S7422** Step 2

- 1 Aliquot 250 µl serum or plasma into 1.5 ml microcentrifuge tube.
- 2 Add 250 µl [5% Sulfosalicylic acid (w/v), 500 µM L-Norleucine] to 1.5 ml microcentrifuge tube. Note: if less than 250 µl serum or plasma is available, can use as little as 100 µl and add reagent in 1:1 (v/v) ratio. i.e., if using 150 µl serum, would add 150 µl [5% Sulfosalicylic acid (w/v), 500 µM L-Norleucine].

☒ L-Norleucine **Millipore Sigma Catalog #N8513-100mg**

☒ 5-Sulfosalicylic acid dihydrate BioXtra >=99.0% **Sigma – Aldrich Catalog #S7422**

- 2.1 To make SSA solution, weigh 5 g sulfosalicylic acid into graduated cylinder, add 6.6 +/- 0.1 mg L-Norleucine, then fill to 100 mL with Biochrom lithium loading buffer. Stir briefly on magnetic stir plate until all solids are fully dissolved. Store at 4°C.


- 3 Vortex each on high speed for 5-10 seconds, and then store in 4°C for 10 minutes.

- 4 Centrifuge 1.5 ml tubes at 10,000 rcf, 4°C for 5 minutes. ☒ 10000 x g

Expected result

- 5 Transfer supernatant to centrifuge filter tubes, being careful not to disturb pellet or floating lipid layer.

 750µL Nonsterile Micro-Centrifugal PVDF Membrane Filters, 0.2µm pore size **Thermo**  
**Fisher Catalog #F2517-5**

- 6 Centrifuge filter tubes at 10,000 rcf, 4°C for 5 minutes.  10000 x g Discard filter and store filtrate at –80°C until analysis.

Expected result