SYKAM CHROMATOGRAPHY

STANDARD OPERATION PROCEDURE

Serum and plasma sample preparation by acidic protein precipitation

Draft

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1. Version History

Modification correlated to last version should be indicated in document.

Date	Status / Version	Author	Modifications
23.03.2016	0.1	Jasper Hölzer	Created

2. Scope of Application

Sample Preparation for serum and plasma by acidic protein precipitation.

3. Description

This procedure describes the preparation of serum and plasma samples for the qualitative and quantitative determination of free amino acids by acidic protein precipitation. The proteins get denaturized by sulfosalicylic acid and are than removed from the sample. For reliable quantitative results for instable amino acids it is important that the sample preparation and measurement is carried out as fast as possible. If the duration between sample preparation and measurement is longer than a few hours the prepared sample should be store at -20 °C or colder. Otherwise instable amino acids will decompose or will be transduced. Glutamine transduces to glutamic acid in a very short time. The results will change significantly within 24 hour storage at room temperature. Further important instable amino acids are: asparagine and aspartic acid, cysteine, homocysteine and arginine-succinic acid.

4. Required Resources

4.1 Equipment

- centrifuge for micro centrifuge tubes, 1.5 ml
- refrigerator at 0-5 °C or similar cooling device for micro centrifuge tubes and vials
- freezer at -20 °C or lower if required for sample storage
- vortex stirrer for micro centrifuge tubes and vials
- adjustable micro pipets (e.g. Eppendorf pipets) with maximum volumes of 1000 µl, 200 µl and if internal standard should be used also 10 µl

4.2 Chemicals

- deionized water or higher grade
- 5-sulfosalicylic acid-dihydrate (CAS 5965-83-3) for analysis or higher grade (e.g. VWR 20678.187)

4.3 Consumables

- glass bottle with screw cap, volume: ≥ 50 ml
- micro centrifuge tubes, 1.5 ml
- sample Vials for auto sampler of amino acid analyzer
- tips for micro pipets
- Sykam sample dilution buffer for physiological samples (SDB/Li) (Sykam Cat. No.: S000015)
- Amino acid standard mix for physiological samples
  - Sykam Standard Type PH-S: 29 amino acids (concentration in µmol/ml)
    Adjusted to serum and plasma samples (Sykam Cat. No.: S000033)
  - Sykam Standard Type PH: 35 amino acids (concentration in µmol/ml)
    Universal physiological standard (Sykam Cat. No.: S000033)
  - Sykam Standard Type PH-X: 40 amino acids (concentration in µmol/ml)
    Extended physiological standard (Sykam Cat. No.: S004301)

* one per sample which are prepared simultaneously

5. Procedure

5.1 Pre-preparation:

1. Sulfosalicylic Acid Solution 10 %

   Prepare a solution of 5-Sulfosalicylic acid in deionized water with a concentration of 10 mass% and 1 µmol/ml Norleucine as internal standard. Therefore solve 2.33 g of 5-Sulfosalicylic acid-dihydrate (MW 254.22 g/mol) and 2.62 mg Norleucine (MW 131.17 g/mol) in 20 ml deionized water. For different preparation volumes take care to calculate with the water free 5-Sulfosalicylic acid (MW 218.19 g/mol) instead of the dihydrate.

2. Internal Standard Solution

   For preparation of internal standard solutions see SOP: Preparation procedure for Amino Acid Standard Additions QM-4101.

All these solutions except internal standard solutions can be stored for about 2 years. For advised storage time of internal standard solution see related SOP.

5.2 Sample preparation:

- Pour 800 µl plasma or serum into a micro centrifuge tube.
- Add 200 µl sulfosalicylic acid solution (10 %) and shake well with vortex mixer.
- Cool down the solution in the refrigerator at about 4 °C for 30 min.
- Centrifuge the cooled sample (e.g. 10 min at 14,000 rpm).
- Dilute an aliquot of supernatant liquor with the same amount of sample dilution buffer (e.g. 500 µl sample with 500 µl sample dilution buffer).
- If using an internal standard (e.g. norleucine) which is not included to the SSA solution dilute the sample as follows. Pour an aliquot of supernatant liquor in a vial and add 10 µl of internal standard solution (e.g. norleucine) with a concentration of 10 µmol/ml and dilute with the same amount of sample dilution buffer minus 10 µl (e.g. 500 µl sample add 10 µl internal standard solution diluted with 490 µl sample dilution buffer).
- These solutions are now ready for injection. When using aforementioned dilution the internal standard concentration is 100 nmol/ml Norleucine.
- These prepared samples should be stored at -20 °C if not measured directly. Also under these conditions instable amino acids will decompose slowly hence store for only a few days. For more precise results prepare a standard with additional amino acids like glutamine at the same time as the sample and store it in the same way.

6. Additional Information

In case of massive retention time variations in the beginning of a sample chromatogram compared to a standard measurement check the pH value of the injected sample. The pH value should be in the range of about 2.00 to 2.20.

The dilution factor for the described sample preparation is 2.5.

In Case of ambiguities don't hesitate to contact labsupport@sykam.com