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Version 1

Colorimetric sulfo-phospho-vanillin (SPV) method for analysis of lipids in mucin V.1

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1 Prepare the standard sample solution

1. Prepare the solvent, chloroform: methanol=2:1;
2. Mix cholesterol in solvent at predetermined concentration, for instance 5mg/ml or 10 mg/ml.
3. Vary volume of the standard sample to assign different amount of cholesterol in different tubes.

2 Prepare the mucin samples

1. Dissolve the mucins in water at a predetermined concentration;
2. Vary the volume of mucins to assign different amount of mucins in different tubes;

3 Evaporating solvent in both standard samples and mucin samples**4 Measure background absorbance**

1. Add 100 ul concentrated sulfuric acid into each tube and incubating at 90 C for 10 min (on a dry heating bath);
2. Cooling to room temperature and measuring background absorbance at 540nm;

5 Measure the absorbance after color development

1. Prepare the sulfo-phosphoric-vanillin acid agent: 0.2 mg vanillin per ml 17% phosphoric acid) for color development;
2. Add 50 ul sulfo-phosphoric-vanillin acid agent for color development;
3. Measuring absorbance at 540 nm after 5 min of color development.