Colorimetric sulfo-phospho-vanillin (SPV) method for analysis of lipids in mucin Version 2

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Abstract

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Protocol

Step 1.
Prepare the mucin samples

Dissolve the mucins in water at a predetermined concentration;

Step 2.
Extraction of lipids in mucin sample solution

1. For each 1 ml of sample solution, add 3.75 ml 1:2 (v/v) chloroform: methanol and vortex well;
2. Then add 1.25 ml chloroform and vortex well;
3. Add 1.25 ml dH2O and vortex well;
4. Centrifuge at 1000 RPM for 5 min to give a two-phase system (aqueous top, organic bottom);
5. Recover the bottom phase with a Pasteur pipette and store in tubes.

Step 3.
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;
3. Vary volume of the standard sample (1 ul, 2 ul, 4 ul, 8 ul, 16 ul and 24 ul)to assign different amount of cholesterol in different tubes.

Step 4.
Evaporating solvent in both standard samples and mucin samples

Step 5.
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;

Step 6.
Measure the absorbanece after color development
1. Prepare the sulfo-phosphoric-vanillin acid agen: 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.