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

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Abstract

Citation: Xueying Zhong Colorimetric sulfo-phospho-vanillin (SPV) method for analysis of lipids in mucin. **protocols.io** dx.doi.org/10.17504/protocols.io.eghbbt6
Published: 08 Feb 2016

Protocol

Step 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.

Step 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;

Step 3.
Evaporating solvent in both standard samples and mucin samples

Step 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;

Step 5.
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.