Protocol 2. The general phenol-sulfuric acid assay for carbohydrate [2]

• Sensitivity: ~ 1-60 μg glucose in 200 μl • Final volume: 1.4 ml (~ 30 μM -2 mM)[a]

Method

1. Prepare the reagent by dissolving phenol in water (5% *w/v*).[b]
2. Mix samples, standards, and control solutions (200 μl containing up to 100 μg carbohydrate) with 200 μl of phenol reagent.
3. Add 1.0 ml of concentrated sulfuric acid rapidly and directly to the solution surface without allowing it to touch the sides of the tube.[c]
4. Leave the solutions undisturbed for 10 mm before shaking vigorously.
5. Determine the absorbances at 490 nm after a further 30 mm.

[a] Aldoses, ketoses, and alduronic acids respond to different degrees. Protein, cysteine, non-carbohydrate reducing agents, heavy metal ions, and azide interfere with this assay. However, it remains useful as a rapid non-specific method for the detection of neutral carbohydrate in column eluates, and is also applicable to solids containing carbohydrate, such as cereal flours, so long as all particles are milled to less than 50 μm diameter. The cysteine-sulfuric acid assay [3] may be used where a sixfold increase in sensitivity is required.

[b] This reagent is stable indefinitely.

[c] The reproducibility of this assay is strongly dependent on the manner of the addition of the sulfuric acid.