Protocol 8. The carbazole assay for uronic acids [11]

• Sensitivity: ~ 0.2-20 µg D-galacturonic acid in 250 µl (~ 4-400 µM)• Final volume: 1.8 ml

Reagents

A Dissolve 0.9 g of sodium tetraborate decahydrate in 10 ml of water and add 90 ml of ice-cold 98% concentrated sulfuric acid carefully to form a layer. Leave undisturbed overnight to mix without excessive heat production. Check it is thoroughly mixed and at room temperature before use.[b]

B Dissolve 100 mg of carbazole (recrystallized from ethanol) in 100 ml of absolute ethanol.[b]

Method

1. Cool the samples, standards, and controls (250 µl) in an ice-bath.
2. Carefully add ice-cold reagent A (1.5 ml) with mixing and cooling in the ice-bath.
3. Heat the mixtures at 100°C for 10 mm.
4. Cool rapidly in the ice-bath.
5. Add 50 µl of reagent B and mix well.
6. Re-heat at 100°C for 15 mm.
7. Cool rapidly to room temperature and determine the absorbance at 525 nm.

[a] Neutral carbohydrates interfere with this assay to a greater (10% on a molar basis for hexoses) or lesser extent (2% on a molar basis for 6-deoxyhexoses). However interference can be reduced by use of appropriate controls as non-uronic acid carbohydrates give significantly different absorption spectra. Cysteine and other thiols increase the response of the assay but large amounts of protein may depress the colour development. Different uronic acids give different responses in this assay.

[b] These reagents are stable indefinitely if refrigerated.