

Removal of free D-galactose (and D-glucose) from milk.

The normal Carrez sample treatment is not performed.

Alternatively, add 1 mL of milk to 4 mL of water and then add 1 mL of freshly prepared sodium borohydride solution (10 mg/mL, dissolved in 50 mM NaOH).

Incubate this solution in a closed plastic tube at 40°C for 30 min. Add 2.5 mL of 0.2 M acetic acid to neutralise the solution and to remove all un-reacted sodium borohydride. Filter the solution through Whatman No. 1 filter paper.

Analyse 0.2 mL of the extract (which is slightly hazy) without any further treatment. The haze in the sample solution is stable in the assay and contributes very little to the background absorbance.

The borohydride reduces all reducing sugars in the milk to their sugar alcohols, i.e. D-glucose to sorbitol, D-galactose to galactitol, and the residual lactose is reduced to lactitol. Borohydride is removed by acidification, and on incubation with β -galactosidase, lactitol is hydrolysed to D-galactose and sorbitol. Galactose is then measured using β -galactose dehydrogenase/ galactose mutarotase in the presence of NAD^+ .

Reagents, additional to those used in K-LACGAR kit, required to perform these analyses are:

1. Sodium borohydride (Sigma S-9125)
2. 50 mM NaOH
3. 0.2 M acetic acid