Ketogluconate Broth

M1324

Ketogluconate Broth is used for identifying bacteria that can utilize a-ketogluconate to form 2-ketogluconate.

Composition**

Ingredients	Gms / Litre
Potassium gluconate	20.000
Potassium dihydrogen phosphate	5.400
Potassium nitrate	2.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 27.4 grams in 1000 ml distilled water. Mix thoroughly. Filter sterilize the medium and aseptically distribute into sterile screw-capped tubes.

Principle And Interpretation

Gluconate oxidation was originally used by Haynes (1) to differentiate the Pseudomonads, but other organisms, mainly those among the *Enterobacteriaceae* , are now known to possess this ability. Gluconate is one of the oxidation products formed from glucose by aerobic microorganisms that metabolize carbohydrates by the Entner-Doudoroff pathway. Bacteria metabolize carbohydrates by either fermentation or oxidation. In fermentation, glucose catabolism involves initial phosphorylation, then a splitting into two triose molecules. However, when glucose is metabolized oxidatively to gluconic acid, no initial phosphorylation occurs and only organisms capable of oxidative metabolism can use potassium gluconate as their sole carbon sources (2,3,4). These oxidative organisms are obligate aerobes (5).

Ketogluconate Broth is used for testing the ability of an organism to oxidize gluconate to 2-ketogluconate, which subsequently accumulates in the medium (6). The basis of the test is the change from gluconate (a nonreducing compound) to 2-ketogluconate (a reducing compound) when tested with a suitable reagent (7).

The medium contains potassium gluconate, which is used as sole carbon source, and potassium nitrate, which is the nitrogen source.

Inoculate heavy inoculum into 1ml of the sterile, dispensed medium. Incubate at 37°C for 48 hrs. Then add 1ml of Benedicts reagent for reducing sugars, place the tube in a boiling water bath for 10 minutes. Observe for the production of a coloured precipitate of cuprous oxide.

Organisms capable of oxidative metabolism use potassium gluconate as their sole carbon source, leading to the accumulation of 2-ketogluconate in the medium. 2-ketogluconate reduces copper sulphate, when heated, to an insoluble cuprous oxide, which is precipitated out as yellow to orange-to-orange red precipitate. The colour produced depends on the amount of 2- ketogluconate accumulated, the greater the amount, the more orange-to-orange red the colour becomes. However, any reducing activity with colours ranging from slight green to deep orange indicates oxidation (8, 5).

Quality Control

Appearance
White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium
Colourless clear solution without any precipitate

Reaction
Reaction of 2.74% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH
6.30-6.70

Cultural Response

M1324: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.(Reaction : On heating in a boiling water bath for 10 minutes after addition of Benedicts reagent)

Organism	Inoculum (CFU)	Growth	Reaction
Cultural Response			
Citrobacter freundii ATCC 8090	50-100	good	positive reaction, green to orange precipitate
Escherichia coli ATCC 25922	50-100	fair-good	negative reaction,blue colour of the reagent is changed
Klebsiella pneumoniae ATCC 13883	50-100	good	positive reaction, green to orange precipitate
Pseudomonas aeruginosa ATCC 10145	50-100	good	positive reaction,green to orange precipitate

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label

Reference

1.Hayens W. C., J. Gen. Microbiol., 1951;5 (5):939-950
2.Hugh R., Leifson E., J. Bacteriol., 1953; 66(1);24-26
3.Pease M., Malcolm J., Chernaik R., Dunlop S., Am. J. Med. Technol.,1698; 34(1): 51-57
4.Sebek O. K., Randles C. I., J. Bacteriol., 1952; 63 (6): 693-700
5.MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Edi., Lippincott, Williams and Wilkins, Baltimore
6.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiol., 8th Ed., American Society for Microbiology, Washington, D.C.
7.Collee J. G., Fraser A. G., Marmion B. P., Simmons A. (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
8.Oberhofer T. R., Manual of Practical Medical Microbiology and Parasitology, New York, John Wiley and Sons. 1985: 172-173

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