TM 2144 - KETOGLUCONATE BROTH

INTENDED USE

For use in identifying bacteria that can utilize a-ketogluconate to form a-ketogluconate.

PRODUCT SUMMARY AND EXPLANATION

Gluconate oxidation was originally used by Haynes 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. These oxidative organisms are obligate aerobes.

Ketogluconate Broth is used for testing the ability of an organism to oxidize gluconate to 2-ketogluconate, which subsequently accumulates in the medium. 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.

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.

COMPOSITION

Ingredients	Gms / Ltr
Potassium gluconate	20.000
Potassium dihydrogen phosphate	5.400
Potassium nitrate	2.000

PRINCIPLE

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

INSTRUCTION FOR USE

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

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : White to cream homogeneous free flowing powder.
Appearance of prepared medium : Colourless clear solution without any precipitate.
pH (at 25°C) : 6.5±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

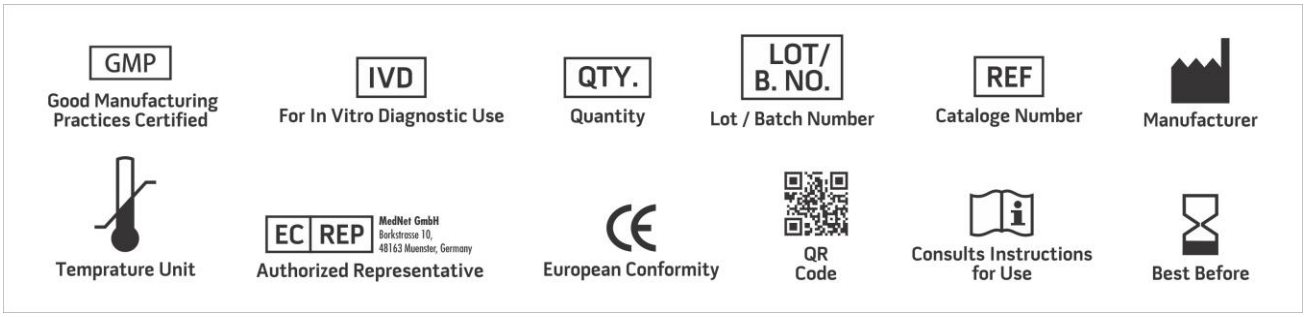
Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Reaction	Incubation Temperature	Incubation Period
Citrobacter freundii	8090	50-100	Good	Positive reaction, green to orange precipitate	35-37°C	18-24 Hours
Escherichia coli	25922	50-100	Fair-good	Negative reaction, blue colour of the reagent is changed	35-37°C	18-24 Hours
Klebsiella pneumoniae	13883	50-100	Good	Positive reaction, green to orange precipitate	35-37°C	18-24 Hours
Pseudomonas aeruginosa	10145	50-100	Good	Positive reaction, green to orange precipitate	35-37°C	18-24 Hours

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL
After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES
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 Sebek O. K., Randles C. I., J. Bacteriol., 1952; 63 (6): 693-700
4. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Edi., Lippincott, Williams and Wilkins, Baltimore
5. 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.
6. Collee J. G., Fraser A. G., Marmion B. P., Simmons A. (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone
7. Oberhofer T. R., Manual of Practical Medical Microbiology and Parasitology, New York, John Wiley and Sons. 1985: 172-173.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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