

Kohn Two Tube HiVeg™ Medium No.2**MV802**

Kohn Two Tube HiVeg Medium No. 2 is used for the identification of members of *Enterobacteriaceae* on the basis of sucrose and salicin fermentation, motility, H₂S (Hydrogen sulphide) production and indole production.

Composition ** :

| Ingredients | Grams/Litre |
|----------------------------------|-------------|
| HiVeg peptone | 10.0 |
| HiVeg hydrolysate | 10.0 |
| Sucrose | 10.0 |
| Salicin | 10.0 |
| Sodium chloride | 5.0 |
| Sodium thiosulphate | 0.016 |
| Disodium hydrogen orthophosphate | 0.09 |
| Bromo thymol blue | 0.02 |
| Agar | 3.0 |

Final pH (at 25°C) 7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters.

Directions :

Suspend 48.13 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 10 lbs pressure (115°C) for 15 minutes. Cool the tubed medium in an upright position.

Principle and Interpretation :

Kohn Two Tube HiVeg Medium No. 2 is prepared by using HiVeg peptone and HiVeg hydrolysate which is free of BSE/TSE risks. This medium is the modification of the medium which Russell (1) first introduced ie Double Sugar Medium. Kohn (2) developed a technique employing two tubes of composite media for study of culture reactions for the identification of the *Enterobacteriaceae*. Gillies (3) made minor modification in Kohn's Media.

Using a straight wire, inoculate with a single stab to about one-third of the depth of the medium. Suspend the two test papers [one is lead acetate paper strip (DD034) and the other Kovac's Reagent strip (DD019)] above the medium by bending and trapping them between the cotton wool plug and the side of the the test tube. Incubate at 37°C for 18-24 hours and examine for motility, H₂S (Hydrogen sulphide) production, sugar fermentation and indole production.

Motility is seen as diffused growth spreading from the line of inoculation. H₂S (Hydrogen sulphide) production is indicated by the blackening of the lead acetate paper strip. Fermentation of sucrose or salicin or both is indicated by the colour change to yellow. Indole formation is indicated by the change in colour of the paper to pinkish red.

Quality Control :**Appearance of powder**

Yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

Product Profile :

| Vegetable based (Code MV)© | Animal based (Code M) |
|--|---|
| MV802 HiVeg peptone HiVeg hydrolysate | M802 Peptic digest of animal tissue Casein enzymic hydrolysate |

Recommended for : The identification of members of *Enterobacteriaceae* on the basis of sucrose and salicin fermentation, motility, H₂S and indole production.

Reconstitution : 48.13 g/l

Quantity on preparation (100g) : 2.07 L

pH (25°C) : 7.4 ± 0.2

Supplement : None

Sterilization : 115°C / 15 minutes.

Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.

Gelling

Semisolid comparable with 0.3% Agar gel.

Colour and Clarity

Green coloured, clear to slightly opalescent gel forms in tubes.

Reaction

Reaction of 4.8 % w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

| Organisms (ATCC) | Fermentation* | Motility | H ₂ S | Indole |
|--|---------------|----------|------------------|--------|
| <i>Proteus vulgaris</i> (13315) | AG or- | + | ± | ± |
| <i>Salmonella</i> serotype Typhi (6539) | - | + | + | - |
| <i>Salmonella</i> serotype Typhimurium (14028) | - | + | ± | - |
| <i>Shigella flexneri</i> (12022) | - | - | - | ± |
| <i>Shigella schmitzi</i> | - | - | - | + |
| <i>Shigella sonnei</i> (25931) | - | - | - | - |

Key : * => Fermentation of Sucrose / Salicin
AG => acid and gas production
+ => positive reaction
- => negative reaction
± => variable reaction

References :

- Russell F.F., 1911, J. Med. Res., 25:217.
- Kohn. J. 1954. J. Path. Bact., 67(1). 286.
- Gillies R.R., 1956, J. Clin. Path., 9(4):368.