

40405 Hippurate Disks

Recommended in qualitative procedures to detect organisms possessing the enzyme hippurate hydrolase, which promotes the hydrolysis of the peptide bond in hippurate, releasing glycine and benzoic acid as end products. The benzoic acid can be detected by using ferric chloride indicator. To detect the glycine with ninyhydrin is possible but any free amino acid will interfere. The disk method is a rapid test useful for presumptive identification of *Gardnerella vaginalis*, *Campylobacter jejuni*, *Listeria monocytogenes* and β -hemolytic group B streptococci.

Contents:

(1 package contains 25 disks)

Sterile filter paper disks (diameter 10mm) impregnated with sodium hippurate.

Directions:

Aseptically place Hippurate Disk in Brain Heart Infusion Broth (Fluka 53286) inoculate with suspect colony. Incubate at 35°C for 48 hours.

Separate the supernatant from the cells by centrifugation. Add 2 ml of ferric chloride reagents to 2 ml of supernatant from the centrifuged culture tubes. Shake well and observe persistence of the precipitate formed even after 10 minutes. Brown flocculants precipitate persisting on shaking after 10 minutes indicates hippurate hydrolysis.

Preparation of ferric chloride reagents:

(12g Ferric chloride, 94.6ml distilled water, 5.4ml concentrated hydrochloric acid)

Give approximately 75 ml of distilled water into a 100 ml graduated flask. Cautious pipette 5.4 ml of HCL to the flask and add 12 g of ferric chloride. Dissolve by warming the flask gently, swirling the contents to mix well. Bring the volume up to 100 ml with distilled water. The solution appears orange in color.

Quality control:

Test Organisms (ATCC)	Growth	Hippurate hydrolysis
<i>Enterococcus faecalis</i> (29212)	+++	-
<i>Streptococcus agalactiae</i> (4768)	+++	+
<i>Streptococcus pyrogenes</i> (19615)	+++	-

Key Hippurate hydrolysis: + = brown flocculants precipitate persisting on shaking after 10 minutes.
- = if any precipitate will be visible it can be dissolved by shaking.

References:

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