

Hippurate Test



Kit used for the identification and determination of hippuricase producing bacteria.

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Hippurate Test Tubes	30 tubes
Hippurate Test Discs	30 discs
Ninhydrin Reagent	3.5 mL
1 Instruction sheet	

Storage condition : Store at 2 - 6°C

• PROCEDURE

1. Take the disc and test tube from the refrigerator and leave it for a few minutes on the bench until it has reached room temperature.
2. Add 0.2 mL of sterile distilled water to a Hippurate Test Tube.
3. Inoculate heavy pure bacterial colonies from fresh culture medium using a sterile loop to the test tube and mix well by using a vortex mixer.
4. Add a Hippurate Test Disc to the tube with organism.
5. Incubate at $36 \pm 1^\circ\text{C}$ for 2 hours.
6. After the incubation, add 3 - 4 drops of the Ninhydrin Reagent to the test tube and mix well.
7. Re-incubate at $36 \pm 1^\circ\text{C}$ for 10 - 30 minutes. Observe the color change of the suspension at 10 minute intervals during the incubation.

**If incubated for more than 30 minutes, false positive reaction may occur.*

• INTERPRETATION

Hippurate Test is a kit used for the identification and determination of hippuricase producing bacteria. Group B Streptococci and some other bacteria (some *Listeria* spp. and *Campylobacter jejuni*.) contain the enzyme hippuricase (hippurate hydrolase) which can hydrolyze hippuric acid to sodium benzoate and glycine. The hydrolysis of hippuric acid is detected by ninhydrin reagent. Ninhydrin is a strong oxidizing agent that deaminates α -amino groups of glycine with the release of NH_3 and CO_2 . The released ammonia reacts with residual ninhydrin to form a purple color.

• INTERPRETATION OF THE RESULTS

The development of a purple-blue color within 10 - 30 minutes indicates a positive reaction. Interpret the results according to the table;

	Reaction
Positive	deep purple / blue color
Negative	no color change / faint blue / gray

• QUALITY CONTROL FOR USE

Appearance : Disc - 6mm paper disc

Reagent - clear

Color : Disc - white (colorless)

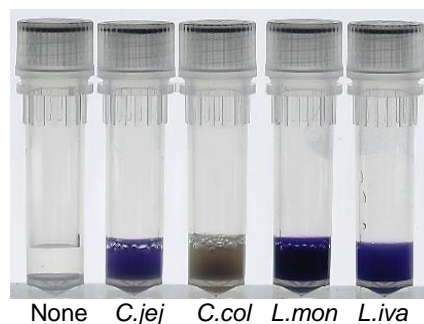
Reagent - light yellow

Incubation conditions : $36 \pm 1^{\circ}\text{C}$ / 2 hours

after addition of the reagent, $36 \pm 1^{\circ}\text{C}$ / 10 - 30 minutes

Microorganism		Hippurate Test
<i>Campylobacter jejuni</i>	ATCC 33291	+
<i>Campylobacter coli</i>	ATCC 33559	-
<i>Listeria monocytogenes</i>	ATCC 15313	+
<i>Listeria ivanovii</i>	ATCC 19119	+

*A faint blue is considered as negative reaction.



• STORE

Store the Hippurate Test at $2 - 6^{\circ}\text{C}$ in its original package tightly closed and use it before the expiry date on the label. Keep away from sources of heat and avoid excessive changes of temperature.

• REFERENCES

1. Finegold S.M., Martin W.J., Scott E.G. In: Bailey and Scott's Diagnostic Microbiology, 5th edition, p. 490. St. Louis, CV. Mosby, 1978.
2. Koneman E.W., Allen V.R.D., Sommers H.M. Color Atlas and Textbook of Diagnostic Microbiology, 2nd ed. 1983.
3. Piot, P. Gardnerella vaginalis. In: Manual of Clinical Microbiology. Lennette E.H., Balowes A., Hausler W.J., Shadomy H.J. Eds. American Society for microbiology. Washington, D.C. 1985.
4. Chapin K.C., Lauderdale T.L. Reagents, Stain and Media: bacteriology. In: Manual of Clinical Microbiology. Murray P.R., Baron E.J., Jorgensen J.H., Teller M.A., Tenover F.C. Eds. American Society for microbiology. Washington, D.C. 2003.

• PACKAGE

Cat. No : MB-88007 Hippurate Test	30 tubes 30 discs Reagent 3.5 mL
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