Ninhydrin 3.5%

REF CK9058

Intended Use
This test is used to determine the ability of bacterial strains to hydrolze hippurate by the action of the enzyme hippurate hydrolase

Background
The hippurate hydrolysis test determines the ability of group B streptococci, as well as other bacteria to enzymatically hydrolze sodium hippurate.
In addition to group B streptococci this method can also be used to identify *Gardnerella vaginalis* and *Campylobacter* spp.
Hippuric acid is hydrolysed by the enzyme hippuricase to glycine and benzoic acid. Ninhydrin evokes a 5 step reaction beginning with the deamination of glycine to form hydrindantin, carbon dioxide and ammonia. A condensation reaction occurs with the hydrintantin, ammonia and residual Ninhydrin to produce the final intense blue/black coloured complex.

Precautions
This product is for in-vitro diagnostic use and should be used by properly trained laboratory professionals. Universal precautions should be taken in the handling, processing and discarding of all materials used to perform the test. Do not use reagents after the expiration date shown on the product label has expired.

Methods
Use a fresh 18-24 hour culture as older cultures could be less metabolically active and results from these may be unreliable.

1. Prepare a dense suspension of the test strain (at least McFarland 4) in 0.25ml of saline
2. Add one hippurate hydrolysis Diatab to the suspension, close and incubate at 35-37C for a minimum of 4 hours or overnight.
3. Following incubation add 5 drops of Ninhydrin 3.5% to the tube, reincubate for no longer than 10 minutes and observe for a colour change.

For a positive reaction a dark blue/black coloured complex will form. With a negative reaction there should be no colour change, a faint colour change should be disregarded.
If results are inconclusive the test must be repeated using a longer incubation period for the hippurate hydrolysis, reincubation of an already completed test will not give a more conclusive result

Results
Positive Reaction – appearance of an intense blue or blue/black within 10 minutes.
Negative Reaction – light grey or no colour change.

Limitations
Plastic tubes should be used as glass may yield a positive result.
Incubation of the sample for more than 30 minutes following the addition of the Ninhydrin Reagent may yield false positive results.

Quality Control
A quality control should be undertaken daily or immediately prior to use.
Bacteria
Positive control-
Streptococcus agalactiae ATCC 12386
Negative control-
Streptococcus pyogenes ATCC 12344

Shelf Life & Storage
The expiry date, storage temperature (fridge) and storage conditions are indicated on the outer package label.

Materials provided
Each pack contains 5 x 3ml dropit bottles containing ready to use Ninhydrin 3.5% reagent.

Materials required but not provided
Sterile loops or needles
Sterile physiological saline
Small tubes

References

Barrow, G.I. & Feltham, R.K.A. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third edition.
Ford, M. Medical Microbiology. Oxford University Press.
Jolly, J.L.S. Minimal criteria for the identification of *Gardnerella vaginalis* isolated from the vagina. J. Clin. Pathol. 1983;**36**:476-478.
Greenwood, J.R. & Pickett, M.J. Salient features of *Haemophilus vaginalis*. J. Clin. Microbio1979;**9**:200-204.
Cacho, J.B. et al. Evaluation of a disk method for detection of hippurate hydrolysis by *Campylobacter* spp. 1989;**27**:359-360.

REF	Catalogue number
LOT	Batch number
	Use by date
IVD	In-Vitro Diagnostic device
Σ	Contains sufficient for <n> tests
	Temperature storage limitations
	Consult instructions for use
	Manufacturer

Issue	Date	Comments
3	29/10/2020	IFU format revision.

CE