Nessler's Reagent

R017

Intended use

Nessler's Reagent is used for determination of Urea (as Ammonia).

Composition**

Ingredients	**-**
Potassium iodide	50.0 g
Distilled water	50.0ml
Final pH (at 25°C)	12.4±0.1

Saturate with mercuric chloride solution until a permanent precipitate just appears. Add 200ml of sodium hydroxide (5mol/ litre). Make the volume to 1 liter with distilled water. Use the clear supernatant as the Nessler's reagent.

**Formula adjusted, standardized to suit performance parameters

Directions

1. Emulsify a 24 hours old culture of organism to be tested for urease test in 0.5 ml substrate in a test tube containing 2% urea.
2. Place the tube in a water bath at 37°C for 3 hours.
3. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes.
4. Read the results after 3-5 minutes after adding Nessler's Reagent. Both negative and control tubes must be absolutely colourless.
5. When isolated colonies are to be examined, the volume of substrate is reduced to 0.3 ml and only one drop of Nessler's reagent is added.
6. For detecting NH_3 production in L-arginine breakdown, Remove a loopful from a 4 day L-arginine culture and place into 0.5 ml of ammonia free distilled water.
7. Add 1 drop of Nessler's reagent. Run the same check on the control

Principle And Interpretation

Bacteria, particularly those growing naturally in an environment exposed to urine may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator. An alternative method is to test for the production of ammonia from urea by means of Nessler's reagent and/or to detect NH3 production due to L-arginine breakdown.

Type of specimen

Used as biochemical reagent in diagnosis.

Specimen Collection and Handling

1. For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The reaction time of ammonia with Nessler's reagent also affects the accurate quantification of NH_3.
2. The result of the Nessler method becomes inaccurate if there are interfering substances such as Cl_2, Cl^-,hardness causing compounds (e.g., Mg^{2+}), and Fe^{2+} in target samples.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Colourless to yellow solution
- **Clarity :** Clear with no insoluble particles.

  Note : On storage of the reagent, precipitate may develop. This will not affect the performance criteria.
- **Reaction :** Reaction of the solution at 25°C. pH :12.4±0.1
- **Test :** Emulsify a 24 hour old culture of organism to be tested for urease test tube containing 2% urea. Place the tube in water bath at 37°C for 3 hours . Remove tube and add 0.1ml of Nessler's reagent and similar amount to the negative control and blank tubes.
- **Results :** A positive reaction is shown by a colour ranging from pale yellow but distinct yellow to dark brown precipitate. A brown colour indicates that L-arginine is degraded in the absence of urease by the arginine dehydrolase system.

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

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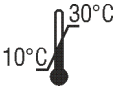

Storage temperature

Do not use if package is damaged

In vitro diagnostic medical device

CE Marking

HiMedia Laboratories Pvt Limited
C-40,21/Y, MIDC, Wagle Ind Area,
Thane(W)–400604,Maharashtra,India

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www.cepartner4u.eu

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