



Nessler's Reagent

R017

Nessler's reagent is used to detect production of ammonia.

Composition**

Ingredients

Potassium iodide	50.0gm
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 litre with distilled water. Use the clear supernatant as the Nessler's reagent.

**Formula adjusted, standardized to suit performance parameters.

Directions

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. Place the tube in a waterbath at 37°C for 3 hours. Remove the tube and add 0.1 ml of Nessler's reagent and similar amount to the negative control and blank tubes. Read the results after 3 - 5 minutes after adding the Nessler's reagent. Both negative and control tubes must be absolutely colourless. 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.

For detecting NH₃ 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. 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 (NH₃) 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 (4) and/or to detect NH₃ production due to L-arginine breakdown .

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

12.3- 12.50

Test

Emulsify a 24 hour old culture of organism to be tested for urease test tube containing 2% urea. Place the tube in waterbath 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. Read results after 3-5 minutes after adding Nessler's reagent.

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.

Reference

- 1) Mackie and MacCartney, 1989, Practical Medical Microbiology, colle J.P., Fraser A.G and Marmino B.P (Eds), 13th ed. Churchill Livingstone, Edinburgh.
- 2) Kauffmann F. and Moeller U., 1995, Acta Pathol. Microbio. Scand., 36:173.
- 3) MacFaddin J., 1980, biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.

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