PRODUCT SPECIFICATION SHEET

KOVACS REAGENT
INDOLE TEST REAGENT

Use

Indole test reagent / Kovacs Reagent is used to detect the production of indole by bacteria growing on media containing tryptophan.

Principle

Kovacs Reagent is used to detect the presence of indole, which is one of the end products from bacterial oxidation of the amino acid, tryptophan. Organisms possessing the enzyme tryptophanase cleave tryptophan, producing three end products: indole, pyruvic acid, and ammonia. The end product indole is produced in aerobic conditions and skatole is produced in anaerobic conditions. Detection of indole indicates tryptophan degradation and can be accomplished by the addition of certain aldehydes to form colored end products. The active ingredient in Kovacs Reagent, p-dimethylaminobenzaldehyde, reacts with indole to form a pinkish-red end product that is highly visible. Amyl alcohol in Kovacs reagent acts as a solvent for indole which then reacts with p-Dimethylaminobenzaldehyde to produce a red rosindole dye. Skatole will also give an indole reaction. Organisms which do not produce the enzyme, produce no color change in the medium when kovacs reagent is added.

The indole test is performed on cultures grown in broth media containing a suitable amount of tryptophan such as casein broth. The use of Kovacs reagent has also been described for combined test media such as Indole-Nitrate Medium (Micromaster DM121), Motility-Indole-Lysine Medium (MIL- Micromaster DM567), Motility-Indole-Ornithine Medium (MIO- Micromaster DM618), and Sulfide-Indole-Motility (SIM- Micromaster DM231) Medium.

Formula

Ingredients	Formula / Litre
p-Dimethylaminobenzaldehyde	50.0 g
Iso-amyl Alcohol	750.0 ml
Hydrochloric Acid, conc	250.0 ml

Reagent Storage And Stability

1. Store the reagents at 2-8 °C. DO NOT FREEZE
2. The shelf life of reagents is as per the expiry date mentioned on the reagent bottle labels.

Precautions

1. For Invitro Diagnostic use only.
2. Observe all standard safety precautions consistent with hazard(s) stated
3. Avoid contact with eyes, skin, or mucous membranes. If contact occurs, wash immediately with copious amounts of water. The reagent has corrosive and flammable liquids; keep away from open flame.

Procedure

A. Conventional Procedure

1. Using a sterile inoculating loop, lightly inoculate 4.0 mL of Tryptone Broth/Tryptone Water (Micromaster DM279) using growth from an overnight, pure culture plate.

2. Incubate at 35°C for 24 to 48 hours. If testing is performed after 24 hours it is recommended that a 2.0-mL portion be removed aseptically for the test. If negative, the remaining broth should be re-incubated for an additional 24 hours and retested.
3. Add five drops (0.5 mL) of Kovac's Reagent (IR002), gently from the side of the tube, to form a ring on the surface of the medium.
4. Check the color of the ring immediately.

B. Microtechnique Procedure (Arnold & Weaver)

1. Dispense 1.0 mL aliquots of Tryptone Broth/Tryptone Water (Micromaster DM279) into small, clean 10x75 mm test tubes. Sterility of tubes is not essential.
2. Preheat broth tubes in a 37°C water bath.
3. Heavily inoculate broth using growth obtained from an overnight culture.
4. Add 4 drops of Kovac's Reagent.
5. Incubate broth cultures in a 37°C water bath from 6 minutes and up to 2 hours.
6. Check intermittently for color change.

Interpretation

Positive: Development of a red color at the interface of the reagent and the broth within 30 seconds
Variable: Orange color at the surface of the medium (usually occurs after 24 hours, re-incubate as indicated)
Negative: No color change (yellow)

- A variable result may occur due to the formation of skatole, a methylated compound that can be a precursor to indole formation
- Some organisms form indole but break it down as rapidly as it is produced and therefore false-negative reactions may occur. This occurs mainly among some Clostridium species
- Other reagents are also available for performing the indole test. Ehrlich's and p-dimethylaminocinnamaldehyde (PACA) are also suitable and in some instances maybe more sensitive than Kovac's Reagent

User Quality Control

Check signs of deterioration. Check the performance of the reagent weekly using 18-24 hour standard ATCC cultures of known indole-positive and indole -negative microorganisms.
The following test strains are recommended:

Organism	**Expected Results**	
Escherichia coli ATCC 25922	Positive	Red color change
Pseudomonas aeruginosa ATCC 27853	Negative	No colour change

Packaging

Product Name : **Kovac's Reagent**
Product Code : **IR002**
Available Pack sizes : **100ml**

Storage & Stability

Kovacs Reagent should be stored at 4°C to 8°C and protected from light. Under these conditions, the reagent has a shelf life of 52 weeks from the date of manufacture.

References

1. Kovacs N. Eine vereinfachte methode zum nachweis der indolbidung durch bakterien. Z Immunitaetsforsch 1928; 55:311-5.
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3. Arnold WM, Weaver RH. Quick microtechniques for the identification of cultures. J Lab Clin Med 1948; 33:1334-7.
4. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I. Washington, DC: ASM, 1992.
5. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Further Information
For further information please contact your local MICROMASTER Representative.

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