



Indole (Kovacs')

INTENDED USE

Remel Indole (Kovacs') is a reagent recommended for use in qualitative procedures for determining the ability of an organism to split indole from the tryptophan molecule.

SUMMARY AND EXPLANATION

Previous tests for the detection of indole production required a xylene extraction procedure to detect small amounts of indole produced from the breakdown of tryptophan. Kovacs utilized amyl alcohol in place of ethyl alcohol to bypass the extraction procedure involved in the Ehrlich-Boehme reagent.¹ Gadebusch and Gabriel reported that substitution of isoamyl alcohol also resulted in greater stability of the Kovacs' reagent.²

PRINCIPLE

Intracellular enzymes collectively called "tryptophanases" mediate the production of indole by hydrolytic activity against the amino acid, tryptophan. Indole combines with dimethylaminobenzaldehyde to form a red compound. The reaction occurs by a condensation process formed by an acid splitting of the protein.³

REAGENTS (CLASSICAL FORMULA)*

Amyl Alcohol (CAS 71-41-0).....	750.0 ml
p-Dimethylaminobenzaldehyde (CAS 100-10-7)	50.0 g
Hydrochloric Acid (Conc.) (CAS 7647-01-0).....	250.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

DANGER! Causes burns by all exposure routes. Flammable liquid and vapor. Breathing vapors may cause drowsiness and dizziness. May be absorbed through intact skin. Aspiration hazard if swallowed. Can enter lungs and cause damage. May cause central nervous system depression. Repeated or prolonged exposure may cause erosion of exposed teeth. Corrosive to metal.

This product is For *In Vitro* Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect from light.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.⁴

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Indole broth (REF R061172), (7) Pipette, (8) Test tube.

PROCEDURE

1. Aliquot 2 ml of indole broth, which has been incubated overnight at 35-37°C, to a separate tube.
2. Dispense 5 drops (0.5 ml) of Indole (Kovacs') reagent down the side of the tube. Shake gently.
3. Observe for color development in the form of a ring interfaced between the broth and the solvent.

INTERPRETATION

Positive Test - A pink-red ring between broth and solvent
Negative Test - No color development

QUALITY CONTROL

All lot numbers of Indole (Kovacs') reagent have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Escherichia coli
ATCC® 25922

Salmonella enterica
serovar *Typhimurium*
ATCC® 14028

INCUBATION

Aerobic, 24h
@ 35-37°C

Aerobic, 24h
@ 35-37°C

RESULTS

Positive

Negative

LIMITATIONS

1. Do not use peptone media containing glucose for indole detection.³
2. Peptone broths (other than tryptophan broth) should be qualified for use in the indole test by testing with a known positive indole-producing organism.³
3. Some organisms form indole but break it down as rapidly as it is produced. A false negative reaction may occur, especially among *Clostridium* species.³
4. The optional pH for tryptophanase activity is one that is slightly alkaline (pH 7.4 to 7.8). A decrease in pH (toward acidity) may result in a false negative or a weakly false positive reaction.³

BIBLIOGRAPHY

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PACKAGING

REF R21227, Indole (Kovacs') 25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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