Kovacs Reagent

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: PL.375

INTENDED USE

For the detection of microorganisms that oxidise the amino acid tryptophan into three major end products: indole, pyruvic acid and ammonia.

SUMMARY AND EXPLANATION

Kovacs reagent was first described by Hungarian-Swiss chemist Erwin Kovacs. Kovacs reagent detects the presence of indole, which indicates tryptophan degradation. Indole production is important in the identification of *Enterobacteriaceae*, a large family of bacteria that includes *Salmonella*, *Shigella* and *Escherichia*. These bacteria are rod-shaped Gram negative facultative anaerobes.

PRINCIPLE OF THE TEST

In certain bacteria, the enzyme tryptophanase breaks down tryptophan into pyruvic acid, ammonia and indole. Indole has the ability to combine with specific aldehydes, which results in the formation of a coloured compound. The active aldehyde in Kovacs reagent, p-Dimethylaminobenzaldehyde, combines with indole to produce a distinct pink-red colour. In the absence of tryptophanase, indole is not produced, and no colour production will be observed. The presence or absence of colour formation is used for bacterial identification.

MATERIALS PROVIDED

- PL.375 Kovacs Reagent 100 ml

Per 100ml solution:

- Kovacs Reagent contains 5g of p-Dimethylaminobenzaldehyde.

MATERIALS REQUIRED BUT NOT PROVIDED

- Inoculating loops
- Filter paper
- Cotton-tipped swabs

STABILITY AND STORAGE

Kovacs Reagent should be stored at 15-25ºC in the original container. Product stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the test.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Process samples in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.
- Any serious incident that occurs in relation to the device should be reported to the manufacturer and the competent authority of the member state in which the incident occurred.

TEST PROCEDURE

Filter paper method:

1. Dispense 1 to 2 drops of Kovacs Reagent onto a piece of filter paper (Whatman No. 1 or equivalent).
2. Using an inoculating loop, smear the growth from an actively growing culture onto the reagent-saturated area of the filter paper.
3. Observe the filter paper for the development of a red colour within 3 minutes.

Swab Method:

1. Dispense 1 to 2 drops of Kovacs Reagent onto the tip of a cotton swab.
2. Touch the tip of the saturated swab to the top of an actively growing colony on the surface of the agar medium.
3. Observe the cotton tip for the development of a red colour within 3 minutes.

QUALITY CONTROL PROCEDURE

Internal quality control of the Kovacs Reagent must be performed regularly on known reference material.

Recommended Quality Control:
Positive - *Escherichia coli* NCTC® 12241/ATCC® 25922* (PLD02)
Negative – *Klebsiella pneumoniae* NCTC® 9633 ATCC® 13883* (PLD04)

INTERPRETATION OF RESULTS

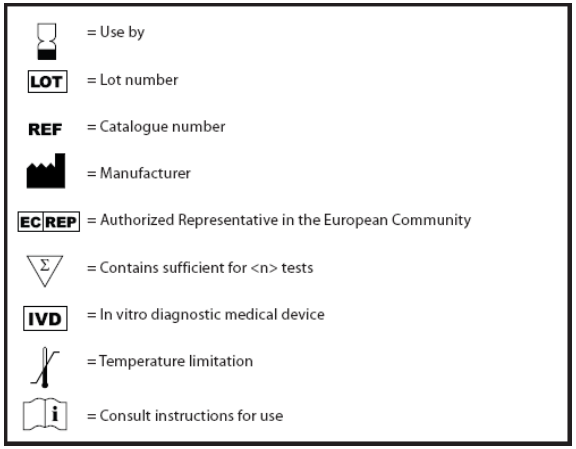
Positive: Development of a red colour within 3 minutes.
Variable: Orange colour (usually occurs after 24 hours, repeat test with a new colony or culture)
Negative: No colour change.

LIMITATIONS OF THE PROCEDURE

- Only experienced personnel should carry out the interpretation of results.
- 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.
- Media must be tested using a known positive and negative control as some types of media may affect the result.

REFERENCES

- Arnold WM, Weaver RH. Quick microtechniques for the identification of cultures. *Journal of Laboratory and Clinical Medicine* 1948; 33:1334-7.
- Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol I Washington, DC: *ASM Press*, 1992.
- Kovacs, N. Eine vereinfachte method zum nachweis der indolbidung durch bakterien. *Z Immunitaetsforsch* 1928; 55: 311-5.



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HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

DANGER	PL.375	H226, H314, H332, H335 P210, P260, P303+P361+P353, P305+P351+P338, P310, P370+P378, P321

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