



## KOVACS REAGENT

- For in vitro use only -

Catalogue No. RK75

Our 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.

Tryptophan is an amino acid that can be oxidized by some bacteria to form three major end products: indole, pyruvic acid, and ammonia. 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.

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-Nitrite Medium, Motility-Indole-Lysine Medium (MIL), Motility-Indole-Ornithine Medium (MIO), and Sulfide-Indole-Motility (SIM) Medium.

The indole test using Kovacs Reagent can be used:

### To aid in differentiation between genera:

1. Separate *Escherichia coli* (+) from members of *Klebsiella* (V-), *Enterobacter* (V-), *Hafnia* (-), *Serratia* (V-), and *Pantoea* (-)
2. *Cardiobacterium hominis* (w+) from *Eikenella corrodens* (-), *Kingella* spp. (-), and *Suttonella indologenes* (-)

### To aid in differentiation between species:

1. *Paenibacillus alvei* (+) from other *Bacillus* spp. (-)
2. *Escherichia coli* (+), *E. hermanii* (+), *E. fergusonii* (+), from *E. vulneris* (-), *E. blattae* (-)
3. *Citrobacter freundii* (-) from *C. koseri* (+) and *C. amalonaticus* and biogroup 1 (+)
4. *Proteus vulgaris* (+), *P. inconstans* (+), *P. rettgeri* (+) from other *Proteus* spp. (-)

5. *Klebsiella oxytoca* (+), *K. ornithinolytica* (+) from other *Klebsiella* spp. (usually -)
6. *Pasteurella dagmatis* (+), *P. dagmatis* subsp. *septica* (V+), *P. multocida* (includes subsp. *gallicida* and subsp. *multocida*) (+), *P. pneumotropica* (+) from *P. haemolytica* (-) and *Actinobacillus ureae* (-)
7. *Paenibacillus alvei* (+) from most frequently isolated species *P. macerans* (-) and *P. polymyxa* (-)
8. *Peptostreptococcus asaccharolyticus* (+) and *P. indolicus* (+) from other *Peptostreptococcus* spp. (-)
9. *Propionibacterium acnes* (+) from other frequently isolated *Propionibacterium* spp. (-)

### Formulation per 100 mL

p-Dimethylaminobenzaldehyde.....	5.0 g
Isoamyl Alcohol.....	75.0 mL
Hydrochloric Acid .....	25.0 mL

### Recommended Procedure

#### A. Conventional Procedure

1. Using a sterile inoculating loop, lightly inoculate 4.0-mL of Casein or Tryptone Broth (Dalynn TT90) using growth from an overnight, pure culture plate.
2. Incubate at 35°C for 24 or 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 reincubated for an additional 24 hours and retested.
3. Add five drops (0.5-mL) of Kovac's Reagent and shake the tube gently.
4. Check for a color change immediately.

B. Microtechnique Procedure  
(Arnold & Weaver)

1. Dispense 1.0-mL aliquots of broth (1% tryptone and 0.3% beef extract) 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 grown on a tryptophan-containing agar or broth.
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 of Results

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, reincubate as indicated)

Negative: No color change (yellow)

- *Although it is less sensitive, Kovac's Reagent is also suitable for the rapid spot test. Refer to the Indole Spot Reagent technical sheet for more information*
- *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-negation 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*

### Quality Control

Organism	Expected Results	
<i>Escherichia coli</i> ATCC 25922	+ve	Red color change
<i>Pseudomonas aeruginosa</i> ATCC 27853	-ve	No colour change

### Storage and Shelf Life

Our 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 indolbildung durch bakterien. Z Immunitaetsforsch 1928; 55:311-5.
2. Reed RW. Nitrate, nitrite, and indole reactions of gas gangrene anaerobes. J Bacteriol 1942; 44:425-32.
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. 3<sup>rd</sup> ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Original: April 2002

Revised / Revisited: October 2014