

Kovac's Reagent

Detection of indole produced due to the breakdown of tryptophan by certain types of bacteria that produce tryptophanase.

REF: BS.1/KR01.050.0050	50 ml	REF: BS.1/KR01.500.0500	500 ml
REF: BS.1/KR01.100.0100	100ml		

CLINICAL SIGNIFICANCE

Detection of indole production by coliforms, is a key feature in differentiation of bacteria. It is used as part of the IMViC procedures. It's useful in separating most strains of *E. coli*, that break down the amino acid tryptophan with the release of indole (indole positive), from members of the *Klebsiella-Enterobacter-Hafnia-Serratia* group which are mostly indole negative.

METHOD PRINCIPLE

Peptone Water is mainly suitable as a substrate in the study of indole production. Peptone used in Peptone Water, is rich in tryptophan content. Other peptones which contain tryptophan can be used to study indole production. Tryptone Water is also recommended by APHA for detection of indole production by coliforms. Kovac's reagent is a biochemical reagent made up of isoamyl alcohol, para-dimethylaminobenzaldehyde (DMAB), and concentrated hydrochloric acid. It is used to determine the ability of the organism to split tryptophan into indole and alpha-aminopropionic acid by hydrolytic activity of bacteria that express tryptophanase enzyme. The indole produced is indicated by formation of a red coloured ring.

REAGENT COMPOSITION

Formulation per 100 ml

p-Dimethylaminobenzaldehyde	5.00 gm
Isoamyl Alcohol	75.00 ml
Hydrochloric Acid	25.0 ml

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the Kovac's Reagent material safety data sheet.

STORAGE, STABILITY AND TECHNIQUE

BioScien Kovac's Reagent is stable until expiration date stated on label when properly stored at 2-8°C away from light. Add 0.2 - 0.3 ml of Kovac's reagent to 5 ml of a 24 - 48 hours old culture of the organism under examination. Observe the formation of a red colored ring which indicates positive indole test within 1-3 minutes.

Deterioration

The color of **BioScien** Kovac's Reagent is greenish yellow colored solution. It's immiscible with water and should be clear with no insoluble particles.

SPECIMEN

Sample consists of bacterial growth obtained from a clinical sample or water sample.

EQUIPMENT REQUIRED NOT PROVIDED

- Inoculating loop
- Sterile test tube with peptone water
- Incubator
- Autoclave

RESULT INTERPRETATION

Positive test result: Formation of a pink to reddish color in the reagent layer on top of the medium within seconds of adding the reagent.

Negative test result: No color change even after the addition of appropriate reagent

QUALITY CONTROL

Organism	Indole production
<i>Enterobacter aerogenes</i> (ATCC 13048)	No red ring formation (i.e. Negative reaction).
<i>Escherichia coli</i> (ATCC 25922)	Red ring formation at the interface of the medium (i.e., Positive reaction).

REFERENCES

1. MacFaddin J., 1980, Biochemical Tests for Identification of Medical Bacteria, 2nd ed., Williams and Wilkins, Baltimore.
2. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and

Wastewater, 21st ed., APHA, Washington, D.C.

3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.

4. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

SYMBOLS IN PRODUCT LABELLING			
	For in-vitro diagnostic use		Number of <n> test in the pack
	Batch Code/Lot number		Caution
	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		