

# Kovacs Reagent

**REF** CK9020

## Intended Use

This test detects tryptophanase production which can be used as an aid in the differentiation of the Enterobacterales and other genera.

## Background

Kovacs reagent is used to detect the presence of indole which is one of the end products of the oxidation of the amino acid tryptophan by bacteria (tryptophanase cleaves tryptophan to produce indole, pyruvate and an Ammonium ion).

The active ingredient of Kovacs Reagent, p-dimethylaminobenzaldehyde reacts with indole to form a pink-red compound.

This reagent is intended for use with Rosco Diagnostica double test Diatabs which include indole detection as a secondary test. The reagent can also be used with the standard tube detection method.

## Precautions

This product is for in-vitro diagnostic use and should be used by properly trained laboratory professionals. Universal precautions should be taken in the handling, processing and discarding of all materials used to perform the test. Do not use reagents after the expiration date shown on the product label has expired.

## Methods

Specimens should be inoculated onto appropriate media to obtain well defined isolated colonies for testing.

## *Tube:*

Inoculate the tryptophan containing broth with the test organism and incubate at 37°C for 24 to 48 hours.

Add 0.5ml of Kovacs reagent and shake gently.

Observe the upper liquid layer for a colour change to a pink-red colour within 3 minutes.

The incubation time can be reduced to 3-4 hours by increasing the cell quantity added and reducing the volume of tryptone water (eg 0.5ml)

## *Rosco Diatabs:*

Prepare a dense suspension (at least McFarland 4) of the strain to be tested in 0.25ml of sterile saline.

Add one diagnostic tablet, close tube and mix. Incubate for 4 hours at 37°C.

After recording the primary test result add 3 drops of Kovacs reagent and observe the upper liquid layer for a colour change to a pink-red colour within 3 minutes.

The incubation period can be extended to 18-24 hours for fastidious organisms, overnight incubation is recommended for 'non fermenters'.

## Results

Positive Reaction – Formation of a pink-red colour surface layer within 3 minutes.

Negative Reaction – Formation of an unchanged or yellow colour surface layer within 3 minutes.

### Limitations

Organisms to be tested must be grown in a tryptophan containing media (for example tryptone water).

Media such as MacConkey Agar should not be used as they contain indicators which could result in the carryover of colour resulting in false positive colour interpretations.

Media containing glucose should not be used as acid production may inhibit indole production.

Only pure cultures should be tested as false positive reactions with mixed cultures (of both indole positive and indole negative) are possible.

### Quality Control

Test colonies must be cultivated in media with adequate tryptophan content and be checked with known positive and negative controls, they should undergo a quality control check daily or immediately prior to use.

Bacteria

Positive control-

*Escherichia coli* ATCC 25922

Negative control-

*Proteus mirabilis* ATCC 12453

### Shelf Life & Storage

The expiry date, storage temperature (10-30°C) and storage conditions are indicated on the outer package label.

### Materials provided

Each pack contains 5 dropper bottles, each dropper bottle contains 3ml of Kovacs Reagent.

### Materials required but not provided









Media and other equipment for subculture.

Sterile loops, needles or swabs

### References

Standards Unit, National Infection Service, PHE. UK SMI, TP19-Indole Test, Issue 4, 03.12.18.

Barrow, G.I. & Feltham, R.K.A. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third edition.

	Catalogue number
	Batch number
	Use by date
	In-Vitro Diagnostic device
	Contains sufficient for <n> tests
	Temperature storage limitations
	Consult instructions for use
	Manufacturer

Issue	Date	Comments
3	03/08/2020	IFU format revision.

