TK PZA KIT

RAPID PYRAZINAMIDE SUSCEPTIBILITY TEST KIT

Catalog No:TK280 Instruction for Use For In Vitro Use Only

Product name:

TK PZA KIT

Intended use:

TK PZA KIT is a kit for rapid determination of susceptibility of mycobacteria to pyrazinamide.

General information:

TK MEDIUM is a culture medium that enables the early detection of mycobacterial growth with its multiple color indicators. In addition, ***TK MEDIUM*** can distinguish mycobacterial growth from contamination. The red color of the medium turns yellow when mycobacteria grow and turns green with the growth of many contaminant bacterial or fungal species, before colonies appear in classical media. The color change can be easily evaluated with naked eye and the tubes can be also monitored with ***MyColor TK***, which is a highly advanced automated incubator and reader. Pyrazinamide is a drug that can show its effect between pH 5.5-6.0. Pyrazinamide is a prodrug. Once it enters into mycobacteria, it is converted to pyrazinoic acid by the enzyme pyrazinamidase. Pyrozinoic acid is the compound which has antimycobacterial activity. At around neutral pH (pH 7.0) pyrazinoic acid, which is rapidly pumped out by the active efflux pump and the drug cannot show its effect. In low pH, this pump cannot work efficiently, and the drug becomes effective. Since it is a drug that can enter macrophages, which are important defense cells against mycobacterial infections, and act in the phagosomal pH of these cells at pH 5.5, pyrazinamide has an important role in completely clearing tuberculosis bacilli from the body. Resistance to pyrazinamide usually occurs as a result of genetic changes that impair pyrazinamidase enzyme activity, resulting in impaired conversion of pyrazinamide to the active drug pyrazinoic acid. [1, 2]

Limitations of the method:

TBR PZA KIT has been developed only to detect pyrazinamide sensitivity of mycobacteria.

Principles of the procedure:

Since pyrazinoic acid formed in bacteria around neutral pH (pH 7.0) is rapidly excreted, pyrazinamide cannot show its effect. Therefore, until now, pyrazinamide sensitivity tests have been performed in media with pH adjusted between 5.5 and 6.0. ***TK PZA KIT*** consists of a special growth control medium with efflux pump inhibitor and a pyrazinamide medium with efflux pump inhibitor. In this way, the test can be performed around neutral pH. The strain to be tested is cultivated on both media. Growth in the control culture medium without pyrazinamide but no growth in the medium with drug indicates that the *tuberculosis bacillus* is sensitive to pyrazinamide. Growth in both control medium and medium with drug indicates that it is resistant to pyrazinamide.[1,2,3]

Ingredients:

TK MEDIUM contains polypeptides, carbohydrates, salts, color indicators and vitamins. In addition to these substances, the ***TK PGC*** control tube contains the active pump inhibitor reserpine, and the ***TK PZA*** tube contains pyrazinamide with reserpine. (white cap)

SUSPENSION TUBE T80 contains 2mm diameter polycarbonate beads in Tween80 solution. (purple cap)

DILUTION TUBE T80 contains Tween80 solution. (purple cap)

Cautions and warnings:

- FOR IN VITRO DIAGNOSTIC USE.
- The tubes should only be opened just before use.
- The caps of the tubes should be closed tightly after inoculation to monitor the change in gas content. The change in gas content will lead to a color change in the medium.
- Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. People who apply these techniques are recommended to have special training in this area. Specimen preparation must be done in a biosafety level II cabinet.
- To reduce the risks of accidental exposure to infectious agents, additional precautions should be taken. At a minimum, specimen manipulation should be done in a contained environment having controlled access, which has a tuberculosis exposure control plan. The locations should have surfaces that can be easily decontaminated using an appropriate topical disinfectant.

General safety precautions:

- Always wear masks and gloves when working with potentially biohazard material.
- Work in a laminar flow cabin, biosafety level II, when pipetting the samples.
- Never mouth pipette.
- A refrigerated centrifuge with airtight swinging buckets is recommended for sedimenting bacteria.
- If spills of the contaminated material occur, disinfect with 2.5% hypochlorite solution.
- Pathogenic microorganisms including Hepatitis B virus and Human Immunodeficiency Virus (HIV) may be present in specimens. Universal precautions and local laboratory guidelines should be followed in handling all items contaminated with blood or body fluids. If a tube leaks or is accidentally broken during collection or transport, use the established procedures in your facility for dealing with mycobacterial spills. At a minimum, universal precautions should be employed.
- Tubes should be discarded in an appropriate manner according to biosafety principles.

Storage instructions:

Store at 2-8°C.

Indications of instability or deterioration:

Do not use the media if a color change to yellow or green is observed prior to inoculation.

Sample preparation:

Mycobacteria grown in any mycobacterial culture medium such as Löwenstein-Jensen, Middlebrook Medium, ***TK MEDIA*** or mycobacteria grown on any medium like ***TK MEDIA*** can be examined with ***TK PZA KIT***.

Recommended Procedures:

Culture should be done in a biosafety level II cabinet.

Materials provided:

The product is offered in sets of 15 in cardboard boxes. Each set consists of 1 ***TK PGC***, 1 ***TK PZA***, 1 ***SUSPENSION TUBE T80*** ve 1 ***DILUTION TUBE T80***.

Necessary materials that are not provided:

- Level II biosafety cabinet.
- Necessary materials and equipment for microbiological cultivation.

Temperature:

The processing of samples and inoculation should be done at room temperature.The incubation of the culture tubes should be done at 37°C.

Time restrictions:

Although the effect of the length of time between processing and inoculation of samples has not been determined, inoculation of the samples immediately after being processed is recommended.

Application:

1. Prepare a 1,0 Mc Farland suspension of mycobacteria in ***SUSPENSION TUBE T80*** from mycobacterial colonies grown on solid media such as Löwenstein Jensen. If mycobacteria are grown in liquid media such as ***TK SLC*** transfer 200 µl of the medium, after vortexing, into ***SUSPENSION TUBE T80*** and mix with the help of vortex for at least 30 seconds.
2. Dilute by transferring 500 µl from suspension in the tube with polycarbonate beads to ***DILUTION TUBE T80*** and vortexing.
3. Write the necessary information about the patient and the sample on one ***TK PGC*** and one ***TK PZA*** tube.
4. Open the caps of the tubes one by one.
5. Add 200µL of diluted mycobacterium suspension to each tube and ensure equal distribution of mycobacteria by pipetting the liquid medium several times.

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6. Close the caps tightly. (**VERY IMPORTANT:** Since the color change in the medium occurs due to the consumption of oxygen in the tube and the accumulation of CO_2, the caps must be tightly closed to prevent gas leakage.)

Incubation:

Place the tubes into a regular 37°C incubator or ***MyColor TK***. If using ***MyColor TK***, enter the necessary information related to the patient and the sample. Select the ***TK PZA*** option in the program.

Evaluation of the results:

Visual evaluation:

Tubes can be easily evaluated visually if an automated incubator-reader, ***MyColor TK*** is not available and the culture tubes are kept in a regular 37°C incubator. The color of the media should be checked visually on a daily basis.

If ***TK PZA*** and ***TK PGC*** both turn yellow, this indicates that the mycobacterium is resistant to pyrazinamide, and if ***TK PZA*** remains red, it is sensitive. Partial resistance may sometimes be present in the mycobacterium strain. Therefore, when the color of ***TK PGC*** turns yellow it is recommended to observe additional 48 hours, before giving the final result in order to see if there is growth in the tubes. This observation time must be followed meticulously. If any of the tubes turn green, it indicates contamination.

Evaluation with *MyColor TK*:

The color change in the tubes is automatically monitored by ***MyColor TK***. When there is growth, result is reported to you by the system.

Important:

Color change in ***TK PGC*** and ***TK PZA*** only allows early detection and prediction of the type of organism growing in the culture. When any type of color change occurs, a smear should be prepared from the tube. After acid-fast staining, the smear should be examined under a microscope to determine whether the microorganism grown in the culture is mycobacteria or another microorganism. The final diagnosis should only be made after examination by experienced personnel.

Quality control:

The following microorganisms are used for quality control and the colours indicated next to them are obtained after cultivation and incubation at 37°C:

Mycobacterium tuberculosis H37Ra:	***TK PGC***	Yellow
Mycobacterium tuberculosis H37Ra:	***TK PZA***	Red
Pyrazinamide resistant mycobacteria:	***TK PGC***	Yellow
Pyrazinamide resistant mycobacteria:	***TK PZA***	Yellow
Uninoculated medium:		Red

Limitations of the procedure:

TK PZA KIT has been developed only to detect pyrazinamide sensitivity of mycobacteria.

Performance characteristics:

Determination of sensitivity of mycobacteria to pyrazinamide using ***TK PZA KIT*** can be done in a shorter time compared to test with conventional media. This period ranges from 2 to 14 days, depending on the growth rate of the mycobacterium species.[1,2]

Shelf life:

Six months.

Bibliography:

1. Zang Y., Mitchison D. The Curious Characteristics of Pyrazinamide. Int. J. Turberc. Lung Dis. 2003; 7(1):6–21
2. Zang Y., Parmar S., Zhonghe S. Conditions that may affect the results of susceptibility testing of Mycobacterium tuberculosis to pyrazinamide. J. Med. Microbiol. 2002; 51:42-49.
3. Kocagöz T., Sarıbaş Z., Şınık G., Öktem S., Aytekin N., Akyar I., Köksalan K. TK PZA: *Mycobacterium tuberculosis*'te Pirazinamit Duyarlılığını Hızlı Saptayabilen Yeni Bir Besiyeri. Trabzon Mikobakteri Günleri. 4-7 Kasım 2009. Trabzon.

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