TK ANTI TB & PNB KIT

RAPID LIQUID CULTURE KIT FOR SUSCEPTIBILITY TESTING TO ANTIMYCOBACTERIAL DRUGS AND IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS COMPLEX AND NON-TUBERCULOSIS MYCOBACTERIA (MYCOBACTERIA OTHER THAN TUBERCULOSIS –MOTT–)

Catalog No:TK250-251 Instruction for Use For In Vitro Use Only

Product Name:

TK ANTI TB & PNB KIT

Intended use:

TK ANTI TB & PNB KIT is a kit used for the rapid determination of susceptibilities of mycobacteria to antimycobacterial drugs and for the rapid differentiation of *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum, M. microti*) species from non-tuberculous mycobacteria (mycobacteria other than tuberculosis –MOTT-).

General Information:

TK MEDIUM is a rapid culture medium with multiple dye indicators that permit early detection of mycobacterial growth. In addition, ***TK MEDIUM*** has the ability to 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. ***TK MEDIUM*** does not contain any radioactive material or fluorescent dye and does not require any scintillation counter, UV light, or other specialized detection systems for evaluation of culture tubes. The color change is easily evaluated by the naked eye or using a low cost but very advanced automated incubator reader, ***MYCOLOR TK.***

When a mycobacterium species is grown from patient samples, it is important to distinguish whether it is *M. tuberculosis* or another mycobacterium species (MOTT). In many cases, MOTT may not be pathogenic to humans and may not require treatment. If the mycobacterium produced is a MOTT species that can cause disease, treatment with drugs other than those used to treat *M. tuberculosis* infection may be necessary. ***TK PNB*** is a ***TK MEDIUM*** type that contains para-nitro-benzois acid (PNB). While PNB prevents the growth of bacteria belonging to the *M. tuberculosis* complex group, it does not prevent the growth of non-tuberculous mycobacteria, thus allowing the two groups to be distinguished from each other. Rapidly increasing drug resistance in the world has increased the importance of susceptibility testing for mycobacteria.
Rapid susceptibility testing can be performed thanks to the media containing antituberculosis drugs in the ***TK ANTI TB & PNB KIT.***

Limitations of the method:

Although rare, PNB can also prevent the growth of some non-tuberculous mycobacterial species. ***TK PNB*** only distinguishes between *M. tuberculosis* complex or MOTT, it does not determine the exact species name of mycobacteria. Additional examinations are required to determine the exact name of the species.

Principles of the procedure:

The growth of mycobacteria in ***TK MEDIUM*** and media containing the antituberculosis drugs isoniazid (***TK INH***), rifampin (***TK RIF***), streptomycin (***TK STR***), and ethambutol (***TK EMB***) and also in ***TK PNB*** are compared. If growth occurs in ***TK MEDIUM*** but not in ***TK PNB***, this indicates that the mycobacterium belongs to the *M. tuberculosis* complex group. If mycobacterium grows in both media, this indicates that the mycobacterium is a non-tuberculous species (MOTT). If growth occurs in ***TK MEDIUM*** but not in media containing antituberculosis drugs, the mycobacteria is susceptible to these drugs; if growth occurs in media containing drugs, it is resistant to the drug in the medium where growth occurs.

Ingredients:

TK MEDIUM contains polypeptides, carbohydrates, salts, dye indicators and vitamins. (white cap)
SUSPENSION TUBE T80 contains Tween 80, polycarbonate beads.

DILUTION TUBE T80 contains Tween 80 solution.

The concentration in tubes containing antimycobacterial substance is as follows:

- ***TK INH*** : Isoniazid 0,05µg/mL
- ***TK RIF*** : Rifampin 1,0µg/mL
- ***TK STR*** : Streptomycin 0,5µg/mL
- ***TK EMB*** : Ethambutol 2,5µg/mL
- ***TK PNB*** : Para-nitro benzoic acid (PNB) 250µg/mL

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 in order to monitor the change in gas content.
- Laboratory methods used to diagnose mycobacteria require special precautions to prevent laboratory-acquired infections. Sample processing should be performed in a level II biosafety room. People performing these procedures should receive special training.
- Additional precautions should be taken to minimize the possibility of laboratory infection. At a minimum, sample processing should be performed in areas where entry and exit are restricted to laboratory personnel. 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% hypo-chloride solution.
- Tubes should be discarded in an appropriate manner according to biosafety principles.

Storage instructions:

Store at 2 to 8 ºC.

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 MEDIUM,*** are suitable for testing with ***TK ANTI TB & PNB KIT***. In order to save time, direct susceptibility testing and typing can be performed in samples found to be acid-fast staining (ARB) positive by microscopy.

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 MEDIUM***, 1 ***TK PNB***, 1 ***TK INH***, 1 ***TK RIF***, 1 ***TK STR***, 1 ***TK EMB***, 1 ***SUSPENSION TUBE T80*** and 1 ***DILUTION TUBE T80***.

Necessary materials that are not provided:

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

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, it is thought that inoculation of the samples immediately after being processed will provide more reliable results.

Application:

If Mycobacteria Culture is in Solid Medium:

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 is grown in liquid media such as ***TK SLC*** transfer 200 µl of the ***TK SLC*** medium, after vortexing, into ***SUSPENSION TUBE T80*** and mix with the help of vortex.
2. Dilute by transferring 500 µl from suspension in the tube with polycarbonate beads to ***DILUTION TUBE T80*** and vortexing.

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3. Write the necessary information about the patient and the sample on one ***TK MEDIUM*** and on one of each tube containing antimycobacterial drugs.
4. Open the caps of the tubes one by one.
5. Transfer 200µL of diluted mycobacterium suspension to each tube. Ensure equal distribution of mycobacteria by pipetting several times.
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.)

It is possible to perform susceptibility testing and typing from clinical samples determined to be AFB+ by microscopy, without growing mycobacteria in culture. Samples prepared with the NALC-NaOH decontamination and concentration method are suitable for culture in ***TK MEDIUM*** and its varieties. The final pH of the sample to be cultured should be between 7.0 and 7.8. It is recommended to use ***MYCOPROSAFE***, ***DECOMICS*** or ***DECOCENT*** for decontamination and concentration. The appropriate pH is easily adjusted in the samples prepared with these kits. Inoculations should be made in a biosafety level II cabinet.

When a direct susceptibility test is performed, inoculation is done in ***TK MEDIUM*** for culture purposes, but since the contamination rate in ***TK MEDIUM*** is higher than the selective medium ***TK SLC***, it is recommended to also do culture in ***TK SLC***. If there is contamination in the tubes containing ***TK MEDIUM*** and tubes with antituberculosis drugs, the procedures can be repeated using mycobacteria cultured in ***TK SLC*****.**

Application of direct susceptibility testing and typing from AFB+ clinical samples:

1. Process the clinical sample for inoculation with decontamination and concentration, preferably using ***DECOMICS***, ***DECOCENT*** or ***MYCOPROSAFE*****.** (If you are using ***DECOMICS***, ***DECOCENT*** or ***MYCOPROSAFE*** follow the instructions for use.)
2. Apply the above-mentioned procedures starting from the third step.

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 antimycobacterial susceptibility testing and typing 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 daily.

The original red color of the media turns yellow, indicating mycobacterial growth. The test should be evaluated when the ***TK MEDIUM*** tube turns yellow. If the ***TK PNB*** remains red at this time, this indicates that the mycobacterial species belongs to the *M. tuberculosis* complex group. If the ***TK MEDIUM*** turns yellow and the ***TK PNB*** also turns yellow, this indicates that the mycobacterium is a non-tuberculous species (MOTT) (see “Limitations of the method”). PNB may slow down the growth of some mycobacterial species. If the ***TK MEDIUM*** color turns yellow and the ***TK PNB*** is red, it is recommended to observe the ***TK PNB*** for another 48 hours before giving a definitive result. If the ***TK MEDIUM*** turns yellow and a tube containing an antimycobacterial drug turns yellow, this indicates that the mycobacterium is resistant to that drug, and if it remains red, this indicates that the mycobacterium is susceptible. Sometimes partial resistance may occur in the mycobacterial strain. Therefore, when the color of ***TK MEDIUM*** turns yellow, it is recommended to observe the tubes containing the drug for another 48 hours before giving the definitive result. If the color turns green in any of the tubes, it indicates contamination.

Evaluation with MYCOLOR TK:

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

Important:

Color change in media only allows early detection of growth 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 colors indicated next to them are obtained after culturing at 37°C:

Mycobacterium tuberculosis H37Ra:	***TK MEDIUM***	Yellow
Mycobacterium tuberculosis H37Ra:	***TK INH***	Red
Mycobacterium tuberculosis H37Ra:	***TK RIF***	Red
Mycobacterium tuberculosis H37Ra:	***TK STR***	Red
Mycobacterium tuberculosis H37Ra:	***TK EMB***	Red
Mycobacterium tuberculosis H37Ra:	***TK PNB***	Red
Mycobacterium smegmatis:	***TK MEDIUM***	Yellow
Mycobacterium smegmatis:	***TK INH***	Yellow
Mycobacterium smegmatis:	***TK RIF***	Yellow
Mycobacterium smegmatis:	***TK STR***	Red
Mycobacterium smegmatis:	***TK EMB***	Red
Mycobacterium smegmatis:	***TK PNB***	Yellow
Resistant M. tuberculosis WHO collection strains		
TK INH, TK RIF, TK STR, TK EMB		Yellow or red, consistent with WHO results
Uninoculated medium:		Red

Limitations of the procedure:

At the end of the decontamination and concentration procedure, the pH of the inoculum should be 7.5 ± 0.2. Inability to neutralize alkaline pH, created by NaOH may change the color of ***TK MEDIUM*** and media with drug from red to purple. This may inhibit or slow down the growth of mycobacteria. In order to adjust the pH, the supernatant should be completely decanted after neutralization with phosphate buffer and precipitation by centrifugation. It is recommended that **DECOMICS**, **DECOCENT** or **MYCOPROSAFE** be used to process the clinical samples to obtain the required pH for inoculation.

Performance characteristics:

Using ***TK ANTI TB & PNB KIT***, the determination of the susceptibility of mycobacteria to antimycobacterial drugs and the differentiation of *M. tuberculosis* complex and MOTT can be done in a shorter time compared to conventional media. This period varies between 2 to 14 days depending on the growth rate of the mycobacterial species. [1,2]

Shelf life:

Six months.

Bibliography:

1. Kocagöz T., A. Alp, A. Albay. A new rapid non-radioactive medium for culturing mycobacteria that also enables visually differentiation of mycobacterial growth from contamination. American Society for Microbiology, 100th General Meeting, Los Angeles. May 21-25, 2000.
2. Kocagöz T., Altın S., Türkyılmaz Ö., Taş İ., Yuca P., Bolaban D., Yeşilyurt E., Öktem S., Aytekin N., Şınık G., Mozioğlu E., Silier T. The efficiency of TK Culture System in the diagnosis of tuberculosis. Diagn Microbiol Infect Dis. 2012 72(4):350-357.

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