SALUBRIS, Inc.

GATEWAY TO HEALTH, WORLDWIDE

TK ANTI TB & PNB KIT®

FOR RAPID ANTIMYCOBACTERIAL SUSCEPTIBILITY TESTING AND MYCOBACTERIAL SPECIES IDENTIFICATION

Catalogue #: TK050 **Instructions for Use** **For In Vitro Diagnostic Use**

Product name:
TK ANTI TB & PNB KIT®

Intended use:
TK MEDIUM® is a differential medium used for rapid determination of mycobacterial growth. Culture results support the rapid diagnosis of tuberculosis. It is intended for in vitro diagnostic use. ***TK PNB®*** (para-nitro benzoic acid) differentiates tuberculosis from non-tuberculosis mycobacteria using para-nitro benzoic acid, which inhibits the growth of mycobacteria belonging to *M. tuberculosis* complex (*M. tuberculosis, M. bovis, M. africanum* and *M. microti*). ***TK ANTI TB & PNB KIT®*** includes ***TK MEDIUM®*** with major antituberculosis drugs INH, rifampin, streptomycin and ethambutol for susceptibility testing of mycobacterial isolates to these drugs.

Summary and explanation of the test:
TK MEDIUM® is a rapid culture medium with multiple dye indicators that permit early detection of mycobacterial growth. Growth in ***TK MEDIUM®*** is compared to the media containing antimicrobial drugs for typing and susceptibility testing. Inhibition by ***TK PNB®*** indicates that the isolate belongs to *Mycobacterium tuberculosis* complex group. Inhibition by any of the antituberculosis drugs indicates susceptibility to this drug.

Limitations:
Some mycobacterial species other than *M. tuberculosis* complex, may be inhibited by ***TK PNB®***.

Principles of the procedure:
The color change in ***TK MEDIUM®*** is based on multiple dye indicators and depends on the metabolites and enzymes produced by different species of microorganisms. The color change occurs long before the colonies become visible. ***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. Determination of susceptibility to antituberculosis drugs is done by comparing the growth in ***TK MEDIUM®*** and ***TK MEDIA®*** containing antituberculosis drugs INH (***TK INH®***), rifampin (***TK RIF®***), streptomycin (***TK STR®***), and ethambutol (***TK EMB®***). Growth on ***TK MEDIUM®*** and inhibition in a drug containing tube indicates susceptibility to this drug. Growth on both tubes indicates resistance. Similarly growth on ***TK MEDIUM®*** and inhibition in ***TK PNB®***, indicates that the bacteria may belong to *M. tuberculosis* complex group (see limitations above). Growth on both of these tubes indicates that the isolate belongs to a species of mycobacteria other than tuberculosis (commonly abreviated as MOTT).

Ingredients:
SUSPENSION TUBE T80® contains Tween 80, glass beads of 1mm diameter.
TK MEDIUM® contains polypeptides, carbohydrates, salts, dye indicators and vitamins. Additionally, the following ***TK MEDIA®*** contain antibacterial as indicated:

- ***TK PNB®***: Para-nitro benzoic acid (PNB) 750µg/mL.
- ***TK INH®***: INH 0.2µg/mL.
- ***TK RIF®***: Rifampin 1.0µg/mL.
- ***TK STR®***: Streptomycin 2.0µg/mL.
- ***TK EMB®***: Ethambutol 7.5µg/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. The change in gas content will lead to a color change in the medium.
- ***TK MEDIUM®*** is a pH sensitive medium. Therefore the pH of the inoculum should be carefully adjusted (to approximately 7.4 ± 0.2) before inoculation. This can easily be achieved using the standard sodium hydroxide-N- acetyl-L-cysteine (NaOH-NALC) decontamination and concentration procedure. It is recommended that the decontamination and concentration kit, ***MYCOPROSAFE®,*** be used in the processing of samples, before the samples are inoculated to ***TK MEDIUM®***. ***MYCOPROSAFE®*** is available separately for this purpose.
- 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 biological safety 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 laminary 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.
- 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 is leaking 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 to 8ºC.

Indications of instability or deterioration:
Do not use the media if a color change to yellow or green is observed prior to inoculation.

Specimen collection and preparation for analysis:
Mycobacteria grown in culture media are suitable for testing with ***TK ANTI TB & PNB KIT®***. Alternatively typing and susceptibility testing can be done directly from AFB smear positive samples. Samples prepared using the NALC-NaOH decontamination and concentration procedure can be used directly for typing and susceptibility testing using ***TK ANTI TB & PNB KIT®***. The final pH of the sample prepared with this method should be within 7.4 ± 0.2. It is recommended that ***MYCOPROSAFE®*** be used to process the samples. This process guarantees the appropriate pH for inoculation.

Recommended Procedures:
Susceptibility testing and typing of a mycobacterial isolate grown in culture:
Apply the whole procedure in a biosafety level II cabinet. Take all precautions to prevent contamination.

1. Write the necessary information about the patient and the sample on the tubes that will be used for susceptibility testing and typing.
2. Suspend mycobacteria grown in ***TK SLC®,*** by pipetting the fluid at the bottom of the tube several times. The tip may be gently smeared on the surface of the medium to suspend more mycobacteria if necessary.
3. Take 200µL of suspension obtained and transfer it ***SUSPENSION TUBE T80®***. Vortex the tube for at least one minute to obtain a homogeneous suspension of turbidity of approximately 1.0 Mc Farland. Repeat these steps if necessary.
4. Transfer 500µL of the suspension into the ***DILUTION TUBE T80®***, close the cap securely and vortex to mix.
5. Transfer 200µL of suspension into each of ***TK MEDIUM®, TK INH®***, ***TK RIF®***, ***TK STR®***, ***TK EMB®*** and ***TK PNB®***. Put the inoculum at the top end of the medium and allow it to slowly migrate downward, wetting the entire surface of the medium.

It is possible to determine susceptibility and to perform typing directly from AFB+ samples without previous isolation of mycobacteria by culture. Samples prepared using the NALC-NaOH decontamination and concentration procedure are suitable for inoculation to ***TK MEDIA***®. The final pH of the sample prepared with this method should be between 7.0 and 7.4. It is recommended that ***MYCOPROSAFE***® be used to process the samples. This process guarantees the appropriate pH for inoculation. Inoculations should be done in a biosafety level II cabinet. It should be remembered that the contamination rate in ***TK MEDIUM***® may be higher than ***TK SLC***®. Inoculation to ***TK SLC***® is recommended if susceptibility testing and typing will be done directly from the clinical sample. If ***TK MEDIUM***® and media containing antituberculosis drugs are contaminated, ***TK SLC***® may isolate mycobacteria without contamination and allow repeated susceptibility testing after pimary isolation.

Susceptibility testing and typing directly from AFB+ clinical samples:

1. Process the clinical sample with the NaOH-NALC decontamination and concentration method, preferably using ***MYCOPROSAFE***®. Follow the package insert instruction of ***MYCOPROSAFE***® for this purpose.
2. Write the necessary patient and sample information on the tubes that will be used for susceptibility testing and typing.
3. Transfer 200µL of decontaminated concentrated sample into each of ***TK MEDIUM***®, ***TK INH***®, ***TK RIF***®, ***TK STR***®, ***TK EMB***® and ***TK PNB***®. Put the inoculum at the top end of the medium and allow it to slowly migrate downward, wetting the entire surface of the medium.

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.

Evaluation of growth:

Visual evaluation:

TK MEDIUM® 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.

A change in color from red to orange and then to yellow will indicate mycobacterial growth. The color of inoculated tubes may be compared to an uninoculated ***TK MEDIUM***® to detect earlier color change. The color change will usually occur before colonies become visible. This is especially likely when the number of colony-forming units (cfu) is high in the inoculated sample. A color change to yellow within 48 hours may indicate contamination with gram-positive bacteria. A color change to yellow between day 3 and day 5 may indicate a rapid-growing species of mycobacteria. A color change to yellow after the 5th day will indicate slow-growing mycobacteria including *M. tuberculosis*.

A change in color from red to green will indicate contamination with either fungi or gram-negative bacteria. In mixed cultures containing both contaminants and mycobacteria, the color will first change to green due to the activity of rapid-growing contaminants and then to yellow due to the activity of mycobacteria.

Evaluation using ***MYCOLOR TK***®:

The evaluation of color changes in culture tubes is automatically followed by ***MYCOLOR TK***® and predictions for growing microorganisms are made accordingly. The color of all culture tubes and growth curves can be easily visualized on the screen. Refer to the user guide of ***MYCOLOR TK***® for this purpose.

Important:

Color change in ***TK MEDIA***® 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 surface of the media or from the fluid collected at the bottom of the tube. After acid-fast staining, the smear should be examined under a microscope for the presence of acid-fast bacilli and other contaminating organisms. **The final diagnosis should only be made after examination by experienced personnel.**

Evaluation of susceptibility to antituberculosis drugs:

When the color of ***TK MEDIUM***® changes to yellow, indicating mycobacterial growth, evaluate the color change in ***TK MEDIA***® containing antituberculosis drugs. If there is a similar color change in any of these tubes, it indicates resistance to this antituberculosis drug. If there is no color change this will indicate susceptibility. Infrequently, a portion of the mycobacterial population may be resistant to an antituberculosis drug. In this case, the resistant portion will require more time to change the color of the media containing the antituberculosis drug. If a color change in an antituberculosis drug containing medium occurs within two days after growth is observed in ***TK MEDIUM***®, this isolate should be reported as resistant to the antituberculosis drug.

Mycobacterium tuberculosis complex and MOTT differentiation:

Growth in ***TK MEDIUM***® and no growth in ***TK PNB***® indicate that the mycobacteria belong to the *M. tuberculosis* complex. Simultaneous growth on both media indicates that the growing mycobacteria do not belong to *M. tuberculosis* complex. However a few species (like *M. brumae*, *M. chitae*, *M. murale*) may also be inhibited by PNB. It should be remembered that these mycobacteria do not normally cause infections in healthy, immunocompetent people and they are very rarely isolated from clinical samples.

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 may shorten the duration of time required to obtain results.

Materials provided:

Sterile ready-to-use ***TK ANTI TB & PNB KIT***® in transparent screw capped tubes (16mm in diameter).

Necessary materials that are not provided:

Biosafety level II cabinet; materials and equipment needed for microbiological culture inoculations.

Quality control:

Quality control of this kit is done by the manufacturer using *Mycobacterium tuberculosis* H37Ra, *Mycobacterium kansasii* ATCC 12478, and quality control antituberculosis drug resistant mycobacterial isolates.

Limitations of the procedure:

Direct susceptibility testing and typing using ***TK ANTI TB & PNB KIT***® can only be applied to samples which are identified to be AFB+ by microscopy. In this case, at the end of the decontamination and concentration procedure, the pH of the inoculum should be 7.4 ± 0.2. Inability to neutralize alkaline pH, created by NaOH may change the color of ***TK MEDIA***® from red to purple and inhibit or slow down the growth of mycobacteria (it is recommended that ***MYCOPROSAFE***® be used to process the clinical samples to obtain the required pH for inoculation). The pH of the processed sample may be too high if the supernatant is not completely eliminated after spinning with the phosphate buffer. This may inhibit the growth of mycobacteria in culture media.

Performance characteristics:

When doing susceptibility testing and typing using ***TK ANTI TB & PNB KIT***® over 95% agreement in susceptibility testing and typing with conventional tests.[1,2]

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. Bicmen C., G. Senol, M. Coskun, N. Florat, T. Kocagoz. Comparison of TK and Löwenstein-Jensen media in antimycobacterial susceptibility testing for *Mycobacterium tuberculosis* by using the proportion method: A preliminary study for a new medium. ECCMID. 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, U. K. May 10-13, 2003.

Manufacturer:
SALUBRIS, Inc.
Baldwin Park I
12 Alfred Street, Suite 300
Woburn, MA 01801, U.S.A.
Tel: (800) 326-1484
Fax: (617) 249-0803
Website: www.salubrisinc.com

Authorized representative:
SALUBRIS
16, avenue de Beauval
92380 Garches – FRANCE
Tel: +33 147 41 28 88

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TK 050

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