TK SLC

RAPID, DIFFERENTIAL AND SELECTIVE MEDIUM FOR CULTURING MYCOBACTERIA

Catalog No: TK221-222 | Instruction for Use | For In Vitro Use Only

Product Name:

TK SLC

Intended use:

TK SLC is a rapid, selective and differential mycobacterial culture medium. It makes an important contribution to the diagnosis of patients suspected of tuberculosis.

TK SLC is used for primary isolation of mycobacteria from samples. ***TK SLC*** contains antibacterials and antifungal, which makes it more selective for mycobacteria, by inhibiting the growth of other types of microorganisms.

General Information:

TK SLC is a culture medium that enables the early detection of mycobacterial growth with its multiple color indicators. In addition, ***TK SLC*** 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. The color change is easily evaluated by the naked eye or using a low cost but very advanced automated incubator reader, ***Mycolor TK.***

Clinical samples, like sputum, contain many microorganisms of normal flora that overgrow mycobacteria in many types of culture media including ***TK SLC***. Therefore, such samples should be decontaminated with the NaOH-NALC (Sodium Hydroxide-N-Acetyl-L-Cysteine) method. (***MYCOPROSAFE*** is a kit containing all the necessary materials for this decontamination and concentration method to be carried out easily and safely. We recommend using ***DECOCENT***, which provides an improved Kubica method, or ***DECOMICS***, which eliminates the need for centrifugation, thus reducing the processing time from 45 minutes to 23 minutes. Some microorganisms other than mycobacteria may survive after the application of this procedure. The antimicrobials included in ***TK SLC*** inhibit the growth of many of these microorganisms and improve the probability that mycobacteria will be isolated.[1-5]

Limitations of the method:

Some microorganisms other than mycobacteria may be resistant to antimicrobials included in ***TK SLC*** and may grow in this medium. Although most contaminant organisms (fungi and Gram-negative bacteria) turn the color of ***TK SLC*** from red to green, gram positive bacteria (e.g. streptococci) may change the color to yellow as with mycobacteria. For that reason, the type of microorganism growing in ***TK SLC*** should always be evaluated by microscopy for the presence of acid-fast staining microorganisms.

Principles of the procedure:

The color change in ***TK SLC*** 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 in conventional media like Löwenstein Jensen. ***TK SLC*** 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.

Ingredients:

TK SLC contains polypeptides, carbohydrates, salts, dye indicators, vitamins and antimicrobials:

- Polymixin B
- Piperacillin
- Amphotericin B
- Nalidixic acid
- Trimethoprim

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. (***TK SLC*** is a pH sensitive medium. Therefore, the pH of the inoculum should be carefully adjusted to approximately 7.5 ± 0.2 before inoculation. It is recommended to use ***TiBO***'s decontamination and concentration kits to adjust the pH appropriately.)
- Laboratory procedures involving mycobacteria require special equipment and techniques to minimize biohazards. Specimen preparation should be performed in a level II biosafety room. People who apply these techniques are recommended to have special training in this area.
- Additional precautions should be taken to reduce the risks of accidental exposure to infectious agents. 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 potential biohazard material.
- Work in 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-chlorite 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 to 8ºC.

Indications of instability or deterioration:

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

Sample preparation:

Samples prepared with ***TiBO***'s decontamination and concentration kits are suitable for inoculation on ***TK SLC***. The final pH of the sample should be 7.4±0.2. Tibo's decontamination and concentration kits ensure the appropriate pH required for culture. Samples taken from sterile body areas such as cerebrospinal fluid (CSF) can be cultured directly in ***TK SLC*** without any further processing.

Recommended procedures:

Inoculations should be performed in a biosafety level II cabinet.

Materials provided:

Each cardboard box contains 150 tubes.

Necessary materials that are not provided:

- Biosafety level II cabinet.
- Materials and equipment needed for microbiological culture inoculations.

Temperature:

The processing of the samples and inoculation should be done at room temperature. Incubations should be performed at 37°C.

Time restrictions:

Although the effect of the length of time between processing and inoculation of the samples has not been determined, inoculation of the samples immediately after being processed may increase the chance of recovery of mycobacteria.

Application:

Inoculations should be performed in a biosafety level II cabinet.

1. Write down patient information on the tube containing ***TK SLC.***
2. Remove the tube cap.
3. Inoculate 500µL of decontaminated and concentrated sample (Samples from sterile body cavities can be cultured directly). Transfer the sample with a sterile pipette tip.

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4. Close the cap tightly. (this is very important since the color change in the medium is partly dependent on the consumption of oxygen and production of CO_2).
5. Vortex.
6. Place the tube into a regular 37°C incubator or ***Mycolor TK***.
7. If using ***Mycolor TK***, enter the necessary information related to the patient and the sample.

Evaluation of results*:*
Visual evaluation:

Even without the automatic incubator-reader ***Mycolor TK***, the ***TK SLC*** can be kept in a regular 37°C incubator and evaluated visually. In this case, the color of the media should be checked visually on a daily basis. A change in color from red to yellow will indicate mycobacterial growth. Orange indicates possible growth and yellow indicates definite growth. A color change to yellow within 48 hours may indicate contamination with Gram-positive bacteria, as mycobacteria generally do not multiply this quickly. 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 usually 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.
***Important*:**
Color change in ***TK SLC*** 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 medium. After acid-fast staining, the smear should be examined under the microscope for the presence of acid-fast bacilli and other contaminating organisms. **The final diagnosis should only be made after examination by experienced personnel.**
Quality control:
The following organisms are used for quality control and the following color changes are obtained after incubation at 37°C:

Mycobacterium tuberculosis H37Ra:	Yellow
Mycobacterium smegmatis:	Yellow
Escherichia coli:	Red
Candida albicans:	Red
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 SLC*** from red to purple. This can slow down or completely prevent the growth of mycobacteria. It is recommended to use ***TiBO***'s decontamination and concentration kits for sample processing and thus ensure the appropriate pH. When using ***Mycoprosafe*** , In order to properly adjust the pH, the supernatant should be completely decanted after neutralization with phosphate buffer and sedimentation by centrifugation.

Performance characteristics:
The detection time for mycobacteria in ***TK SLC*** depends on the mycobacterial load in samples inoculated. With acid fast bacilli positive samples the time required for culture growth is usually between 5 to 18 days.[1,2] When compared to Löwenstein Jensen medium (LJ) the average time required for growth detection in ***TK SLC*** is approximately half the time required for LJ.
Shelf life:
Nine 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. Bicmen C., M. Coskun, G. Senol, N. Florat, T. Kocagoz. Comparison of TK and Löwenstein-Jensen Media for primary culture of Mycobacteria: A preliminary study for a new medium. ECCMID. 13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, U. K. May 10-13, 2003.

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