IVD MIBI

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Tb-Stain

Stain kit (cold staining) for the microscopic determination of mycobacteria

Principle

Tb-Stain is a staining kit for the microscopic examination of mycobacteria based on a modification of the Ziehl-Neesen method. Using this cold stain obviates the usual requirement for heating the carbol-fuchsin solution and thus largely prevents the formation of phenol fumes. Acid-resistant bacteria, e. g. tuberculosis bacteria, have a highly lipid-rich, wax-like cell membrane. It prevents release of the dye, which has been taken up during staining, after acid treatment (hydrochloric acid in alcohol). Acid-fast mycobacteria exhibit red staining on microscopic visualization while non-acid-fast microorganisms show the color of the counterstain.

Reagents

If stored at the recommended storage temperature, the reagents are stable until the expiry date printed on the label. After opening, contamination-free solutions are stable for at least 4 months. Always keep bottles properly closed.

At storage temperatures below 15 °C, dye precipitates may form. These can be dissolved by heating the solution to 60 °C for 4 hours (with periodical mixing) in a water bath.

Sputusolve may gas out when exposed to heat and light. To prevent expansion of the bottle (danger of bursting!), loosen cap immediately after delivery to avoid build-up of internal pressure, and store bottles upright. Avoid using reagents at temperatures below 20 °C.

Risks and Safety

Please observe the necessary precautions for use of laboratory reagents and body fluids; as well as possibly also of microbiological samples. Applications should be performed by expert personnel only. Follow the national and laboratory internal guidelines for work safety and infection control. Wear suitable protective clothing and disposable gloves while handling.

It is important to ensure effective protection against infection according to laboratory guidelines.

SDS ID International
www.sds-id.com

For additional safety information please refer to the information on the label and the corresponding Safety Data Sheet (SDS).

The safety settings were made according to legal guidelines. If there are differences in the labeling or the safety information between the label and SDS, the details of the SDS are valid.

Download by QR-Code or link:

www.sds-id.com/100144-9	(Tb-Stain - Sputusolve)
www.sds-id.com/100145-8	(Tb-Stain - Carbol fuchsine)
www.sds-id.com/100146-7	(Tb-Stain - Destaining Solution)
www.sds-id.com/100147-6	(Tb-Stain - Malachit green)

Contents / Main Components

003831-...	Cont.	NaOCl ~5 %, NaOH ~7.5 %
003832-...	Cont.	6 g/l C.I. 42510/42520, 40 g/l C_6H_5OH
003833-...	Cont.	Hydrochloric acid 0,75 % in C_2H_5OH.
003834-...	Cont.	2 g/l C.I. 42000

003830-6001	**KIT**	**Tb-Stain**	
003831-1010:	R1	1× 1.0 L	Tb-Stain Sputusolve (Concentrate)
003832-0500:	R2	1× 500 mL	Tb-Stain Carbol fuchsine Solution
004101-1010:	R3	1× 1.0 L	Tb-Stain Destaining Solution (HCl-Ethanol)
003834-0500:	R4	1× 500 mL	Tb-Stain Malachit green oxalate Solution

The staining kit is for about 250 stains.

The solutions are also available as individual solutions in various container sizes.

In addition required / recommended

Immersion oil, mounting medium, slides etc.

Reagent Preparation

R1a: Sputusolve working solution 15 % (Dilution 15 : 100):

Approach volume:	100 mL	500 mL	1000 mL
Sputusolve Concentrate	**15 mL**	**75 mL**	**150 mL**
Aqua dest.	**85 mL**	**425 mL**	**850 mL**

Mix and keep dark.

Notes

Use only contamination-free materials. Observe the applicable safety precautions.

Equipment

Microscope, staining rack, centrifuge, microscope slides, tubes, Aqua dem. The use of stainer is possible.

Sample Material

Bacteriological material such as sputum, imprints, rinses, effusions, pus, exudates, fine needle aspiration biopsies (FNAB), liquid and solid cultures, histological sections.

Sputum is preferentially used to diagnose Tb as it mostly contains high numbers of tuberculosis bacteria (> 10^4/ml). The minimum sample volume is approx. 2 mL. Preferably process diagnostic material the same day or store in a refrigerator for max. 24 hrs.

To release the Tb bacteria from the sputum, i.e. to extract them from the enveloping mass of phlegm and cells, the specimen is treated with Sputusolve. The organic material is dissolved while sparing the acid- and alkali-fast tuberculosis bacteria for the greater part. Other mostly undesired germs are largely destroyed.

Produktinformation Tb-Stain (Kaltfärbung)
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Procedure

Sample Preparation

Sputum

To separate mycobacteria from mucus and cellular structures, the sputum should be pretreated with Sputusolve. The contained hypochlorite oxidatively dissolves the organic material and gently releases the mycobacteria.

Sample processing (in the centrifuge tube):		
Sample		**1 Part**
Sputusolve 15%		**3 Part**
Close and shake vigorously	10 min	
Centrifuge at 3000 ... 4800 RPM (U/min)	20 min	
Pour off liquid supernatant. Prepare smear from sediment. Let smear air-dry.		

Histological sections:

Deparaffinize and re-hydrate sections as usual (decreasing ethanol series). Skip pre-treatment with Sputusolve for formalin-fixed samples.

Other sample material:

After suitable enrichment step, prepare, air-dry and heat fix smears.

Fixation

Smears are fixed by passing slides over a Bunsen burner flame (two to three times, avoid exposure to extreme heat, e. g. scorching)

Alternatively fixing can be performed for 20 min at 100-110 °C in a drying cabinet or on a heating plate/slide warmer. Intense heat exposure diminishes the stainability of cells.

Staining on a staining rack

Align slide with fixed smear horizontally.	
Tb-Stain 2 (Carbol fuchsine)	**5 min**
Cover completely, then rise with tap water until no more dye clouds form.	
Tb-Stain 3 (Destainer)	**15 ... 30 sec ***
Cover completely and allow to react. Immediately rinse with tap water.	
* Reaction time depends on sample thickness.	
Tb-Stain 4 (Malachite green)	**1 ... 2 min**
Cover slide completely.	
Rinse carefully with running tap water	**10 sec**
Air-dry over night or at 50 °C in a drying cabinet.	

Histological section must not be de-hydrated after counterstaining as dye will be washed out.

Should samples frequently detach from the object slide, prior coating of the glass slides with an albumin-glycerin mixture is recommended (product information and albumin-glycerin reagent on request).

During staining, take strict care to prevent spreading acid-resistant bacilli from one object slide to another.

If you wish to detect Tb bacteria by growth in culture, use only a 5% Sputusolve solution (fill 50 mL Sputusolve concentrate up to 1000 mL with demineralized water). To prevent irreversible damage to the cells, add 1 N HCl before inoculation.

Staining in the automatic stainer

Slides to drain well between the staining steps to avoid an unnecessary procrastination of reagent.

Slides with fixed smear.	Time:	Station:
Tb-Stain 2 (Carbol fuchsine) *	**5 min**	**1**
Rinse with tap water	45 sec 5	
Tb-Stain 3 (Destaining)	**15 sec**	**2**
Rinse with tap water	15 sec 5	
Tb-Stain 4 (Malachite green)	**60 sec**	**3**
Rinse with tap water	10 sec 5	
Air drying overnight at station 6 or drying in a drying chamber at 50 °C.		
* Exchange the solution in station 1 after approx. 12 runs or as required.		

Analysis

Bright-field microscopical examination of the stained smear for min. 5 min with 100× oil immersion objective. If properly stained, the acid-fast mycobacteria appear clearly red against an amorphic, light-green background.

Diagnosis

Positive test result: "acid-fast bacilli detected".

Negative test result: "acid-fast bacilli not detected"

No statement can be made whether the acid-fast bacilli are tuberculosis bacteria (Mycobacterium tuberculosis) or other mycobacterial species. Likewise, viable (active) bacteria cannot be distinguished from dead (inactive) bacteria. After detection of acid-fast bacilli in the sample material, follow-up examinations in a special laboratory are required.

Diagnostics

Only authorized and sufficiently trained personnel is to make diagnoses. Valid and proper nomenclature is to be used.

Select and perform follow-up tests using established/certified methods.

Proper controls should be included with every sample run to exclude false results.

Infection Prevention

Observe the applicable accident prevention regulations and the currently valid hygiene and disinfection guidelines for tuberculosis-related work. Reliable destruction of any potentially present mycobacteria must be ensured. When working with liquid samples, covering the work space with disinfectant-soaked cellulose tissue is recommended. Processes that may cause aerosols to form (e. g. removing lids/caps from tubes) must be performed in a biological safety cabinet (BSC) with bacteria filter. Also use only tightly sealed containers for centrifugation due to the risk of aerosol formation. Waste (supernatant from centrifugation, used centrifugation tubes/vials, object slides, etc.) is considered infectious and must be treated and disposed of accordingly.

Notes

Classifications

Not for human diagnostics.

Support / Information service

For methodological and technical support, please contact us by E-Mail at support@bioanalytic.de.

Periodically check for updates of this product information on our website.

Feedback

Information from users can be reported to support@bioanalytic.de.

Suggestions for further developments will be considered.

Entsorgung

Please observe all applicable laws and regulations of your country.

Dispose of used or expired solutions according to local laws and regulations.

Inside the EU, regulations - in their respective valid national versions - on the basis of Council directive 67/548/EEG on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances apply. Decontaminated packaging can be disposed of as household waste or recycled unless otherwise regulated.

Dispose of inactivated infectious material following official regulations.

Ordering information

All reagents are available separately (also in other container sizes), as are Löffler's Methylene Blue and the Albumin-Glycerol Solution, if required.