

# TB-STAIN HISTO KIT



IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

Three-reagent kit for staining acid-fast bacteria in histology sections acc. to Ziehl-Neelsen

## INSTRUCTIONS FOR USE



<b>BASIC UDI number</b>	385889212HPC30708STARVF		
<b>EMDN code</b>	W01030708		
<b>REF</b>	<b>Catalog number</b>	<b>Volume</b>	<b>UDI-DI number</b>
TBHK-100T		100 tests	03858890007077

### Intended use and test principle

Many bacterial cells are easily stained with simple stains or with the Gram stain. However, a few bacterial strains, such as Mycobacteria and Nocardia cannot be stained using simple dyes (or, if successfully stained, the results may vary significantly). Cellular wall of the Mycobacteria strain contains waxy substance - mycolic acid. Those are beta-hydroxy carboxylic acids with chains containing up to 90 carbon atoms. Its resistance to acidity is associated with mycolic acid chain length. In order to stain such strains, a higher concentration of dye or a longer period of heating is required. However, once stained, the dye is even more difficult to remove from the cells. Those bacteria are called acid resistant because they maintain their primary color even after decolorization using acid alcohol (Carbol Fuchsin). Early laboratory diagnosis of tuberculosis is based on the interpretation of stained smears, and one of the best diagnostic methods is analyzing sputum sample under microscope. The most common and renowned method used for detecting the tuberculosis bacteria is staining according to Ziehl-Neelsen. This method uses Carbol Fuchsin as the main dye, acid alcohol as decolorization medium and Methylene Blue solution as contrasting dye. BioGnost's TB-Stain Hot kit contains TB Carbol Fuchsin reagent, two packages of TB Decolorizer and Methylene Blue Loeffler reagent.

### Product description

- **TB-STAIN HISTO KIT** - Three-reagent kit in 4 packages. For staining acid-fast bacteria in histology sections according to Ziehl-Neelsen

The kit contains:	for 100 tests (TBHK-100T)	Storage temperature
TB Carbol Fuchsin reagent	30 mL (TBC-OT-30)	15-25°C
TB Decolorizer	2 x 30 mL (TBD-OT-30)	15-25°C
Methylene Blue Loeffler reagent	30 mL (MBL-OT-30)	15-25°C

### Additional reagents and materials that can be used in this method

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- Infiltration and embedding agent, such as BioGnost's granulated paraffin BioWax Plus 56/58, BioWax 56/68, BioWax Blue
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C, FF, 37 or Tropical Grade

### Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 µm thin slices and mount on a VitroGnost microscope slide

### NOTE

Apply the reagent so it completely covers the section.

### Sample staining procedure

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Add TB Carbol Fuchsin reagent (≥5 drops)	30 min
	Note: place the section in an incubation jar in order to avoid drying of the section	
6.	Rinse in distilled/demineralized water and dry using filter paper	
7.	Add TB Decolorizer (≥5 drops)	5-15 sec
8.	Rinse under tap water	3 min
9.	Add Methylene Blue Loeffler reagent (≥5 drops)	30 sec
10.	Rinse in distilled/demineralized water	3 exchanges, 10 sec each
11.	Rinse under tap water	2 min
	Note: in order to make the stain intensity greater, the section can immediately be covered with BioMount Aqua reagent and VitroGnost cover glass	
12.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
13.	Dehydrate in 100% alcohol (Histanol 100)	2 min
14.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with VitroGnost cover glass.

### Result

Acid – fast bacteria – red  
Nuclei and background – blue

## Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

## Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid an incorrect staining result, it is advised to use a positive and negative control.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

## Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

## Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product label.

## References

1. Ziehl, F. (1882): Zur Färbung des Tuberkelbacillum. Deutsche Medizinische Wochenschrift, V8, pg 451.
2. Neelsen, P. (1883): Zentralblatt für die Medizinischen Wissenschaften, V21, pg497
3. Madison, B. (2001): Application of stains in clinical microbiology. Biotech Histochem 76 (3): 119–25.
4. Ryan, K.J., Ray, C.G. (editors) (2004). Sherris Medical Microbiology (4<sup>th</sup> ed.). McGraw Hill.

### Warnings and precautions regarding the materials contained in the product:

	H225	Highly flammable liquid and vapor.
	H226	Flammable liquid and vapor.
	H314	Causes severe skin burns and eye damage.
	H301	Toxic if swallowed.
	H311	Toxic if on skin.
	H331	Toxic if inhaled.
	H341	Suspected of causing genetic defects.
	H373	May cause damage through prolonged or repeated exposure.
	P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
	P233	Keep only in original container.
P280	Wear protective gloves/protective clothing/eye protection/face protection.	
P302+P352	IF ON SKIN: wash with plenty of water.	
P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.	
P308+P311	IF exposed or concerned: call POISON CENTER/doctor.	

TBHK-IFU\_ENV4, 25.02.2026., IŠP

 Manufacturer	 Batch code	 Consult Instructions for use	 Contains sufficient for <n> tests	 Contains hazardous substances
 Date of manufacture	 Catalogue number	 Caution	 European conformity	 Fragile, handle with care
 Use-by date	 Temperature limit	 <i>In vitro</i> diagnostic medical device	 Unique device identifier	

 **BioGnost Ltd.**  
Medjugorska 59, 10040 Zagreb, Croatia, EU, www.biognost.com

Version	Description / reason for change	Date
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	25.02.2026.