

GRAM-HUCKER STAIN

Principle

Gram-Hucker staining is the most commonly used staining in microbiology to differentiate Gramnegative Gram-negative bacteria according to the retention it presents to the dye. The microorganisms stained with the first dye (Crystal Violet) are called Gram-positive. Microorganisms stained with contrast dye (Safranin O) are Gram-negative. The Gram-Hucker Stain Kit (in dropper) is composed of all the reagents involved in this Staining.

<u>Material</u>

Biological samples (sputum, pus, cultured bacterial cells).

Reagents

Code	Description
256649	Gram-Hucker's Staining Kit (Droppers) for clinical diagnosis (*)
251622	Safranine O (C.I. 50240) for clinical diagnosis (*)
251086	Ethanol absolute for clinical diagnosis (*)
251002	Immersion Oil for clinical diagnosis (*)

Component of the kit

Code	Description	
251803	Alcohol-Acetone 7:3 for clinical diagnosis ^(*)	
251774	Lugol's Liquor with 0,4% of Iodine (diluted) for clinical diagnosis (*)	
252531	Gram-Hucker's Safranine O solution for clinical diagnosis (*)	
252532	Gram-Hucker's Crystal Violet Oxalate solution for clinical diagnosis (*)	

All these components can also be purchased separately.

Preparation of solutions

• If Safranin O (C.I. 50240) is used, the Gram-Hucker solution should be prepared as indicated below:

Safranin O 0.25 g Ethanol Absolute 10 ml Water (s.q.m.) 100 ml



Procedure

1. Fix the microbial smear by heating carefully not to burn the preparation. Make short passes by controlling the temperature of the slide by hand back contact.

- 2. Cover the preparation with Violet Cristal Oxalate Gram-Hucker solution for 1 minute.
- 3. Wash excess dye gently with running water.
- 4. Cover the preparation with Lugol Liquid for 1 minute.
- 5. Rinse again with water.
- 6. Decolorize, drop by drop and no more than 1 minute with 7: 3 Alcohol-Acetone.
- 7. Rinse again with water.
- 8. Cover the preparation with Safranine O solution according to Gram-Hucker for 1 minute.
- 9. Wash with water.
- 10. Let it dry.
- 11. Observe the microscope with immersion objective

<u>Results</u>

Gram-positive bacteria	Blue violet
Bacterias Gram-negativas	Red-pinkish

Technical note

The color of the dropper can change over time. Does not affect staining. The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the manufacturer's instructions and the software must be observed.

Sample preparation

All samples should be treated according to the state of the technology. All samples must be unambiguously labeled.

Diagnostics

Diagnosis should be established only by authorized and qualified persons. Each application should involve appropriate controls to rule out erroneous results.

Storage

The staining solution should be stored at + 15 to + 25 ° C.

Expiration

The product stored at the indicated temperature and in a tightly closed container is usable until the expiration date indicated on the package.



Notes on use

In order to avoid errors, the staining must be carried out by specialized personnel. For professional use only. The national directives on safety at work and quality assurance must be complied with.

Advise on disposal of waste

Solutions used and expired solutions should be disposed of as hazardous waste and local waste disposal regulations must be observed. If further questions are asked about disposal, they may be processed through E-Mail: <u>info.es@itwreagents.com</u>. Inside the EU are valid the requirements based on Council Directive 67/548/EEC on the approximation of the laws, regulations and laws, regulations and administrative provisions relating to the classification, packaging and labeling of dangerous substances in the relevant version.

Classification of hazardous substances

Observe the classification of dangerous substances on the label and the information on the safety data sheet.

Manufacturer

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(*) Sanitary product for In Vitro Diagnostics

