

## LUGOL'S LIQUOR WITH 5% OF IODINE (CONCENTRATED)

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### Principle

Lugol's liquor is an aqueous solution of iodine and potassium iodide. It acts as a mordant in Gram-Hucker staining. A mordant is a substance that allows or enhances the staining of a biological sample by a dye. In the case of Gram-Hucker staining, the dye is usually crystal violet and the role of iodine as a mordant is essential for the proper functioning of the stain.

At a higher concentration, 5%, it has a wide use in protozoology from use for rapid fixation to general staining of human intestinal protozoa. Show starch, glycogen and other polysaccharides.

This concentration is used as a stock solution to prepare, by dilution, the Lugol's Liquid solution for use in Gram staining.

### Material

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

### Reagents

Codes	Description
252532	Gram-Hucker's Crystal Violet Oxalate solution
251803	Alcohol-Acetone 7:3
252531	Gram-Hucker's Safranin O solution

### Procedure

Gram staining uses biological samples (sputum, pus, cultured bacterial cells), usually prepared as smears and fixed with methanol or flame. After staining with crystal violet, the preparation is brought into contact with a 0,4 % solution of Lugol's liquid to pass through the cell wall and come into contact with the previously used crystal violet, the preparation is then decolourised and safranin is used as a contrast dye. The preparation can be observed by light microscopy revealing Gram (+) positive (violet-blue) and Gram (-) negative (red-pink) cells.

The procedure for Gram staining is as follows:

1. Fix the microbial smear by heat taking care not to burn the preparation. Make short passes, checking the temperature of the slide by contact with the back of the hand.
2. Cover the preparation with Crystal Violet Oxalate solution according to Gram-Hucker (252532) for 1 minute.
3. Wash under running water.
4. Cover the preparation with the diluted 0,4 % working preparation solution for 1 minute.
5. Rinse again with water.
6. Discolour, dropwise and not more than 1 minute with 7:3 Alcohol-Acetone (251803).

7. Wash again with water.
8. Cover the preparation with the safranin sample solution.
9. Wash with water and allow to dry for microscopic observation.

### **Results**

Gram-positive bacteria are stained bright violet. Gram-negative bacteria are stained pink. There is a possibility of false results due to either handling errors or unusual characteristics of very specific organisms. It is very important to be careful in the fixation process and in the decolourisation process, since in both cases, too much fixation and decolourisation can lead to false negatives due to loss of cell wall integrity and excessive washing of the violet crystal that can occur.

### **Technical note**

The microscope used should correspond to the requirements of a clinical diagnostic laboratory. If an automatic staining device is used, the operating instructions of the appliance manufacturer 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 room temperature

### **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**

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: [info.es@itwreagents.com](mailto:info.es@itwreagents.com). 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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(\*) In Vitro Diagnostic Medical Device

