

GRAM COLOR KIT

DESCRIPTION

GRAM COLOR KIT is a kit for staining micro-organisms that allows them to be differentiated into two categories: Gram-positives (Gram+), which are coloured blue, and Gram-negatives (Gram-), which are coloured red. Combined with direct observation of the cell morphology, this staining constitutes the first level in the taxonomic classification of prokaryotes.

CONTENT OF THE PACKAGES

The reagents are contained in plastic bottles, sealed by thermo-induction and provided with a dropper lid. Each pack contains:

- 1 bottle containing 250 ml of Crystal Violet Solution
- 1 bottle containing 250 ml of Lugol-PVP Solution
- 1 bottle containing 250 ml of Decolourant Solution
- 1 bottle containing 250 ml of Safranin Solution

PRINCIPLE OF THE METHOD

Gram staining is based on the property of Crystal Violet of combining with iodine to form compounds that cannot be decoloured with alcohol or with an alcohol-acetone mixture. Some bacteria have a special affinity for this reaction and, once stained with crystal violet, do not lose the colour if treated with alcohol or alcohol-acetone mixture, thus retaining the blue colouring (Gram-positive bacteria). Others lose the blue colour and are stained by Safranin, taking a red colour (Gram-negative bacteria).

COLLECTION OF SAMPLES

Samples to be subjected to Gram staining are usually clinical material and microbial cultures. The colonies to be subjected to Gram staining must be taken from young cultures (18-24 hours) preferably on an agar medium.

TEST PROCEDURE

Preparation and fixing

On clean slides, make a smear of the culture or pathological material. Leave to dry in the air and fix by heat, passing rapidly over the flame. Do not overheat the sample when fixing. Other fixing methods may be used.

Staining

1. Cover the slide with the Crystal Violet Solution. Wait 1 minute, then rinse gently with water.
2. Cover the slide with the Lugol-PVP Solution. Wait 1 minute, then rinse delicately with water.
3. Decolour with the Decolourant Solution for as long as the preparation releases colour (about 30-60 seconds), then rinse delicately with water.
4. Cover the slide with the Safranin Solution. Wait 30-60 seconds, then rinse delicately with water.
5. Dry.
6. Examine the preparation under the microscope with the objective for immersion.

INTERPRETATION OF THE RESULTS

The Gram-negative micro-organisms appear as red in colour. The Gram-positive micro-organisms appear as blue in colour. The Gram staining makes it possible to distinguish between:

- Gram-negative bacilli from Gram-positive ones;
- Gram-negative cocci from Gram-positive ones;
- Gram-negative coccobacilli from Gram-positive ones;
- Gram-negative diplococci from Gram-positive ones.

QUALITY CONTROL

Each lot of GRAM COLOR KIT is subjected to quality control using a culture of *Escherichia coli* ATCC 25922 for the test for Gram-negative bacteria (red colour) and a culture of *Staphylococcus aureus* ATCC 25923 for the test for Gram-positive bacteria (blue colour).

LIMITS

- Gram staining provides a preliminary identification but does not replace normal cultural studies of the sample.
- Antibiotic therapy may make Gram-positive bacteria more sensitive to decolouration, so that they appear pinkish-red instead of blue.
- Cells taken from young, 18-24 hour cultures have a greater affinity for the stains than cells taken from older cultures.
- Gram staining is altered by the physical destruction of the cell wall or protoplasm. In fact the cell wall of Gram-positive bacteria constitutes a barrier which impedes release of the Crystal Violet-iodine complex from the cytoplasm, and the cell wall of Gram-negative bacteria contains lipids soluble in organic solvents that

permit decolouration of the cytoplasm. Hence, micro-organisms physically destroyed by an excess of heat do not react as expected to the Gram stain test.

PRECAUTIONS

The GRAM COLOR KIT package contains substances classified as hazardous by current legislation. It is recommended that the Safety Data Sheets be consulted on their use. GRAM COLOR KIT is a kit for bacteria staining, only for diagnostic use *in vitro*. It is intended for use in a professional environment and must be used in a laboratory by adequately trained personnel using approved asepsis and safety methods for dealing with pathogenic agents.

CONSERVATION

Store GRAM COLOR KIT at 10-25°C in the original packaging. Keep away from sources of heat and avoid excessive changes of temperature. In such conditions the product GRAM COLOR KIT will be valid until the expiry date shown on the label. Do not use beyond that date. Eliminate without using if there are signs of deterioration (changes in the colour of the solutions or presence of substantial precipitates).

DISPOSAL OF USED MATERIAL

After use, the slides stained with the GRAM COLOR KIT and any material that has come into contact with the sample must be decontaminated and disposed of in accordance with the techniques used in the laboratory for decontamination and disposal of potentially infected material.











BIBLIOGRAPHY

- Kruczak-Filipov, P., and R.G. Shively. 1992. Gram stain procedure, p.1.5.1-1.5.18. In H.D. Isenberg (ed.) Clinical Microbiology Procedures Handbook, vol. 1. American Society for Microbiology, Washington, D.C.
- Murray, P.R. (ed.) 1999. Manual of Clinical Microbiology, 7th ed. American Society of Microbiology, Washington, D.C.

PRESENTATION

Product	Ref	Content
GRAM COLOR KIT	80293	4 x 250 ml

TABLE OF SYMBOLS

 IVD	In Vitro Diagnostic Medical Device	 Do not reuse
	Manufacturer	 Contains sufficient for <n> tests
 REF	Catalogue number	 Fragile, handle with care
	Use by	 Caution, consult accompanying documents
	Temperature limitation	 LOT Batch code



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Rev.3 / 09.05.2017