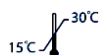


GRAM STAIN PACK

IVD For *in-vitro* diagnostic and professional use only



Store at Room Temperature

INTENDED USE

Gram Stain used for differentiate between gram positive and gram-negative bacteria.

INTRODUCTION

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

PRINCIPLE

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains Blue while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and *counterstaining* with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into CV⁺ and chloride (Cl⁻) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The CV⁺ ion interacts with negatively charged components of bacterial cells and stains the cells Blue.

Iodine (I⁻ or I₃⁻) interacts with CV⁺ and forms large complexes of crystal violet and iodine (CV-I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The

CV-I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV-I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains Blue. and the gram-negative cell loses its Blue. color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.

MATERIALS

MATERIALS PROVIDED

- Crystal Violet.
- Gram Iodine.
- Gram Decolouriser.
- Counterstain – Safranin O.

Note: This package insert is also used for individually packed reagent.

Packaging Content

- REF 8.17.009.0400** (1x100ml Crystal Violet, 1x100ml Iodine Solution, 1x100ml Decolouriser, 1x100ml Safranin O)
- REF 8.17.008.1000** (1x250ml Crystal Violet, 1x250ml Iodine Solution, 1x250ml Decolouriser, 1x250ml Safranin O)
- REF 8.17.009.1000** (1x250ml Crystal Violet, 1x250ml Iodine Solution, 1x250ml Decolouriser, 1x250ml Safranin O)

- REF 8.15.032.0250** (1x250ml Crystal Violet)
- REF 8.15.049.0250** (1x250ml Iodine Solution)
- REF 8.15.051.0250** (1x250ml Decolouriser)
- REF 8.15.125.0250** (1x250ml Safranin O)

STORAGE AND STABILITY

- Store at room temperature.
- Stain Solution is stable up to the printed expiry date.
- Keep the bottles tightly closed to prevent air oxidation.

PRECAUTIONS

- The reagent may cause eye, skin and respiratory tract irritation; so protective clothing should be worn when handling this reagent.
- The reagent is intended for in vitro diagnostic use only.
- Do not use this reagent if the label is not available or damaged.
- Test materials and samples should be discarded properly in biohazards container.
- This reagent is considered toxic, so do not drink or eat beside it.
- Wash hands and test table top with water and soap once the testing is done.

PROCEDURE

1. immerse the heat fixed smears with Crystal Violet and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Flood the smear with Gram Iodine for 2 minutes.
4. Wash with tap water.
5. Decolorize the smear for few second only.
6. Wash thoroughly with tap water.
7. Counterstain with Safranin O for up to 2 minutes.
8. Wash and allow to dry.
9. Examine under microscope using oil immersion objective

RESULTS

- Gram positive organisms (Blue).
- Gram negative organisms (Red).



ATLAS Medical GmbH

Ludwig-Erhard Ring 3

15827 Blankenfelde-Mahlow

Germany

Tel: +49 - 33708 – 3550 30

Email: Info@atlas-medical.com

Website: www.atlas-medical.com

PPI2112A01

Rev C (27.03.2023)

	Catalogue Number		Temperature limit
	In Vitro diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry
	Flammable		