GRAM STAIN PACK

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

Store at Room Temperature

INTENDED USE
For the staining of gram 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 purple 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 purple.

Iodine (I^- or I_3^-) 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 purple and the gram-negative cell loses its purple 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 (250ml)
- Gram Iodine (250ml)
- Gram Decolouriser (250ml)
- Counterstain – Safranin O (250ml)

PROCEDURE

1. Flood the heat fixed smears with Crystal Violet (A) and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Stain with Gram Iodine (B) for 1 minute.
4. Wash with tap water.
5. Decolorize (C) until no further stain runs from the slide.
6. Wash thoroughly with tap water.
7. Counterstain (D) with Safranin O for up to 1 minute.
8. Wash, air dry and examine.

RESULTS

- Gram positive organisms (**purple).**
- Gram negative organisms (**red)**

ATLAS Medical
William James House,
Cowley Road, Cambridge, CB4 4WX, UK
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524

PPI055A01
Rev C (28.10.2015)

REF	Product Reference No.	IVD	For in-vitro diagnostic use.
	Caution.		Store at
	Read product insert before use.		Number of tests in the pack.
LOT	Lot (batch) number.		Manufacturer.
	Expiry date.		Manufacturer telephone number.
	Manufacturer fax number.		