



Product Protocol



Product Name	Gram Stain Kit (Gram Fuchsin Counterstain)
---------------------	---

Product Code	RRSK3-100
---------------------	------------------

Reagents

Crystal Violet (0.5% Aqueous)	1x 50ml
Grams Iodine	1x 50ml
Gram Differentiator	2x 50ml
Gram Fuchsin (Carbol Fuchsin)	1x 50ml

General Information

Number of Tests:	100 (based on bench top staining)
Procedure Time:	18 minutes (approximate)
Shelf Life:	3 Years (from date of manufacture)
Storage:	8-25 °C

Principle

This kit is used to demonstrate Gram Positive and Gram Negative Micro-organisms. The main principle of the technique is the increased thickness, chemical composition and functional integrity of the cell walls of gram positive bacteria, which when they die become gram negative.

Specimen Collection

Smears: Prepare smears on cleaned slides and air dry for 5-10 minutes. Fix in absolute ethanol for 10 minutes.

Tissues: Formaldehyde based fixatives are recommended. Tissues should be processed and embedded in paraffin wax and cut at 5 microns

Protocol for Tissue Sections

1. Dewax sections, hydrate through alcohol and rinse in tap water
2. Stain with filtered crystal violet for 3 minutes
3. Rinse well in distilled water
4. Treat slides with Grams iodine for 3 minute
5. Wash well in distilled water, drain
6. Differentiate with Grams differentiator until no more colour comes out of the preparation (1-5 seconds). Rinse off quickly, sections will be straw coloured (see note 2)
7. Wash well in water
8. Counterstain with Gram fuchsin 1 minute
9. Wash in water and blot dry
10. Dehydrate rapidly through alcohols, clear and mount

Protocol for Smears

Flood slides with distilled water
Stain with crystal violet for 1 minute
Rinse in distilled water
Treat with Grams iodine for 1 minute
Wash in distilled water and drain
Differentiate with Grams differentiator until no more colour comes out of the preparation (1-5 seconds), Rinse off quickly. Sections will be straw coloured (see note 2)
Wash well in water
Counterstain with Gram Fuchsin 30 secs - 1 minute
Wash in water and blot dry
Mount with your usual method and examine microscopically

Results

Gram Positive Organisms	Blue/Black
Gram Negative Organisms	Red/Pink
Nuclei	Red/Pink

Notes

1. Staining times may be modified to provide varying intensities of staining.
2. Do not overdifferentiate as this will result in Gram positive organisms staining red as Gram negative organisms and giving a false result
3. It is recommended that a control slide be used with all sections containing known Gram positive and Gram negative organisms

Stability

If correctly stored the reagents are usable until the expiry date

Disposal

Hazardous Reagents Included, observe local waste disposal regulations

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the member state in which the user and/or the patient is established

