WHITE LABS® TEST KITS

GRAM STAIN
-KIT-

PLEASE READ SAFETY SHEET AND ALL PROCEDURAL INSTRUCTIONS THOROUGHLY BEFORE BEGINNING STAINING PROTOCOL.

YOUR KIT INCLUDES:

- (1) 50mL Gram crystal violet
- (1) 50mL Gram iodine
- (1) 50mL Gram decolorizer
- (1) 50mL Gram safranin
- (2) Gram stain check slides
- (20) sterile pipettes
- (2) pair of laboratory gloves
- Instructions
- Safety sheet

OTHER SUGGESTED MATERIALS:
(Must be purchased separately)

- Alcohol lamp
- Metal or sterile disposable inoculation loop
- Safety glasses
- Kimwipes
- Immersion oil
- Premium microscope slides

BACKGROUND:

A Danish physician, Hans Christian Gram invented the Gram stain in 1884. The invention was born of the need to aid the taxonomy of bacteria. The Gram stain first allows you to separate unidentified bacteria into either one of two groups- Gram positive bacteria or Gram negative bacteria. Other tests, like the catalase reaction test, can then be performed in order to pinpoint the microbe. Another benefit Gram staining provides is increased definition of structure and pattern of the bacteria.

SAFETY:

Please read all safety measures before conducting staining procedure.

PLEASE WEAR APPROPRIATE PROTECTIVE EYEGLASSES AND GLOVES TO MINIMIZE EXPOSURE.

PROTOCOL:

Please note, it is best to designate a small container or bucket for the following staining procedure. The purpose of the container is to prevent staining of the sink or brewery area where the stain is performed. Hold slide over container when applying dye and cool rinses. Dye spillage into container should be diluted with water and disposed of into the sink with copious amount of water.

1. Place sample on clean slide. Dilution with a drop of sterile water may be necessary. It is best to use sterile materials for sample application: sterile inoculation loop or alcohol sterilized metal inoculation loop. Use the sterile pipette tip to spread sample evenly within the sample area.

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The Gram check slides allow staining of up to 6 samples as well as Gram positive and negative control areas. The controls let you monitor your staining techniques. Once you have mastered the staining technique, regular microscope slides can be used for subsequent staining protocols.

2. Let the sample air dry.
3. Fix sample onto slide by passing slide quickly through flame. Fixation protects the sample from dislodging during staining procedure. Sample is now ready for staining.
4. Add drops of crystal violet to sample, making sure sample is covered completely without flooding slide. Allow dye to react for 1 minute.
5. Rinse gently with cool water.
6. Cover sample with drops of Gram iodine and allow to react for 1 minute.
7. Rinse gently with cool water.
8. Apply decolorizer drop by drop onto slide until solution runs clear (approximately 20 seconds.)
9. Rinse gently with cool water.
10. Cover sample with drops of safranin counterstain and allow to react for 30 seconds.
11. Rinse gently for 30 seconds.
12. Blot slide gently (sample side down) onto Kimwipe or other low lint towelette.
13. Let air dry completely.
14. View under 1000x-microscope using immersion oil.

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WHAT YOU SEE:

- Purple/violet cells are Gram positive.
- Red cells are Gram negative.

WHY THEY STAIN THE WAY THEY DO:

In essence, if particular bacteria can retain the crystal violet and iodine complex when challenged with decoloizer, then it's classified as Gram positive. The other (Gram negative) will absorb the counterstain safranin.

Gram positive cells have a characteristically thick, single layout of peptidoglycan that provides structural strength to the cell.

Gram negative cells have a multilayered envelope comprised of the cytoplasmic membrane, the periplasmic space, a think layer of peptidoglycan and a hydrophobic outer membrane.

The crystal violet is a large dye complex that gets trapped in the thick peptidoglycan cell wall. Both Gram positive and negative bacteria can take up the dye; the difference is their ability to retain the dye.

The Gram negative cell wall is partially destroyed by the decolorizer thereby washing out the crystal violet and allowing the safranin counterstain to take hold. The Gram positive bacteria retains most of its cell wall when challenged with the decolorizer as well as the primary crystal violet stain.

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RESULTS:

Yeast is primarily a Gram positive staining organism however, occasionally stains Gram variable (purple-red). Please refer to the chart below for common brewery bacteria.

GRAM POSITIVE		GRAM NEGATIVE	
Organism	Shape	Organism	Shape
Lactobacillus	Rods	*Zygomonas*	Plump rods
Pediococcus	Cocci	*Acetobacter*	Rods
		Pseudomonas	Rods
		Enterobacter	Rods

KIT STORAGE

- Do not store in direct sunlight. Store in cool, dry, well-ventilated area away from incompatible substances.
- Material incompatibilities include acids, strong oxidizing agents, isocyanates, metals, nitrides, peroxides and hydroperoxides, epoxides, nitrogen dioxide and reducing agents.

GRAM CRYSTAL VIOLET (DANGER: POISON)

- Contains ethanol, denatured (20%)
- May be harmful if swallowed. If swallowed and person is conscious, induce vomiting by giving two glasses of milk or water and sticking finger down throat. Call physician. Do not give anything by mouth to an unconscious person.

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- May cause severe eye irritation and possible injury (damage to cornea and conjunctiva). Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Seek medical aid.
- May cause skin irritation. Flush skin with plenty of soap and water for at least 15 minutes. Get medical aid if irritation develops or persists.
- Prolonged inhalation may cause respiratory irritation. First aid for inhalation includes removal from exposure and to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.

GRAM IODINE (DANGER: POISON)

- May be fatal if swallowed. If swallowed and person is conscious, induce vomiting by giving two glasses of water and sticking finger down throat. Call physician immediately. Do not give anything by mouth to an unconscious person.
- In case of eye contact flush eyes with water for 15 minutes. Contact your physician.
- Flush skin with water.

GRAM DECOLORIZER (DANGER: POISON, FLAMMABLE)

- Contains acetone and ethanol, denatured
- May be fatal is swallowed.
- Please use with adequate ventilation.
- Causes eye burns. In case of eye contact flush with water for 15 minutes. Contact your physician.
- Highly flammable. Keep away from heat, sparks and flame. In case of fire extinguish with water, dry chemical, CO_2 or alcohol foam.

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GRAM SAFRANIN (DANGER: POISON)

- Contains ethanol, denatured (10%)
- May be fatal or harmful if swallowed. If swallowed and person is conscious, induce vomiting by giving two glasses of water and sticking finger down throat. Call physician. Do not give anything by mouth to an unconscious person.
- Keep away from heat, sparks and flame.
- Contact with eyes may cause severe irritation and possible eye burns. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower lids. Seek medical aid.
- May cause skin irritation. Flush skin with plenty of soap and water for at least 15 minutes.

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