



Instructions For Use

TGS-IFU

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Twort's Gram Stain Kit

Description and Principle

The Twort's Gram Stain Kit is designed for positive and negative gram bacteria differentiation in formalin fixed paraffin embedded tissues. Gram negative bacteria stain pink to red, gram positive stain blue, and the background is stained light green.

Gentian violet along with Lugol's iodine forms a dye lake staining both gram-positive and gram-negative organisms. Gram-positive and gram-negative bacteria are differentiated due to differences in cell wall composition. Gentian violet-iodine complex is removed from gram-negative bacteria while gram-positive bacteria retain the stain. Twort's Counterstain Solution contains red cations and green anions which simultaneously stain gram-negative bacteria and background tissue.

Expected Results

Gram Positive Bacteria:	Blue
Nuclei and Gram Negative Bacteria:	Red
Background:	Light Green

Kit Contents

1. Gentian Violet Solution	18-25°C
2. Lugol's Iodine Solution	18-25°C
3. Gram's Decolorizer Solution	18-25°C
4. Twort's Neutral Red (Part A)	18-25°C
5. Twort's Fast Green (Part B)	18-25°C

Storage

Suggested Controls (not provided)

Any well-fixed FFPE tissue known to contain both gram(+) and gram(-) bacteria.

Uses/Limitations

For Research Use Only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure:

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Apply adequate Gentian Violet Solution to completely cover tissue section and incubate for 1 minute.
3. Rinse slide in distilled water to remove excess stain.
4. Apply adequate Lugol's Iodine Solution to completely cover tissue section and incubate for 1 minute.
5. Rinse slide in gently running tap water to remove excess iodine.

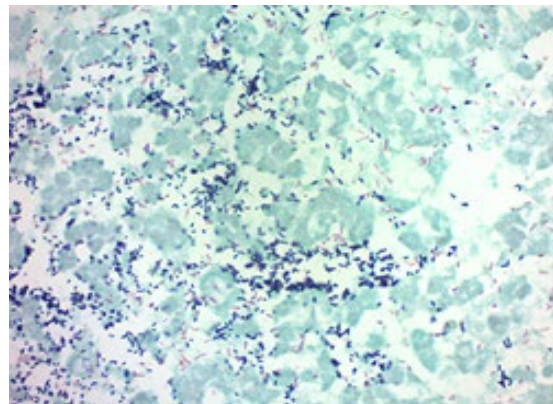


Figure 1. Gram Negative and Positive bacteria demonstrated on artificial control slide of chicken liver.

6. Carefully drip Gram's Decolorizer over tissue until blue color no longer bleeds off section. Note: Decolorization for longer than 5 seconds may remove stain from gram positive bacteria.

7. Rinse slide quickly in gently running tap water. Leave in tap water until working counterstain (next step) is ready.

8. Prepare the following Working Twort's Counterstain solution and mix well.

Working Twort's Counterstain Solution:

(Add components in the order listed)	
30 ml	Distilled Water
*9 ml	Twort's Neutral Red (Part A)
*2 ml	Twort's Fast Green (Part B)

*If initial results/concentrations aren't optimal, adjust concentrations of red and green stain to suit staining intensity preference.

9. Stain slide in Working Twort's Counterstain for 5-10 minutes. Use once and discard.


10. Rinse quickly in distilled water.

11. Blot and/or air dry slide until completely dry.


12. Clear, and mount in synthetic resin.

References

1. Twort F. W. (1924) J. State Med. 32 pg 351

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