



# Instructions For Use

## TCK-IFU

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## Twort's Counterstain Kit

### Description and Principle

The Twort's Counterstain Kit is designed for histological procedures to provide contrast for easier visualization of tissue sections. This kit is also used to counterstain gram-negative bacteria and background tissue in gram stain procedures. **Gram-positive stain is not included in this kit.**

Twort's Counterstain Solution contains red cations and green anions which simultaneously stain nuclei red and cytoplasm green. When used in combination with a gram stain procedure, Twort's Counterstain will stain gram-negative bacteria red, Twort's will not differentiate gram-positive and gram-negative bacteria on its own.

### Expected Results

Nuclei: Red  
Cytoplasm: Light Green

### Kit Contents

1. Twort's Neutral Red (Part A)
2. Twort's Fast Green (Part B)
3. Acetic Alcohol Solution (2%)

### Storage

- 18-25°C  
18-25°C  
18-25°C

### Suggested Controls (not provided)

Any well-fixed tissue, Cytology Smear.

### Uses/Limitations

For In-Vitro Diagnostic 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.

### Prepare the Following Solution Immediately Before Use.

#### Working Twort's Counterstain Solution:

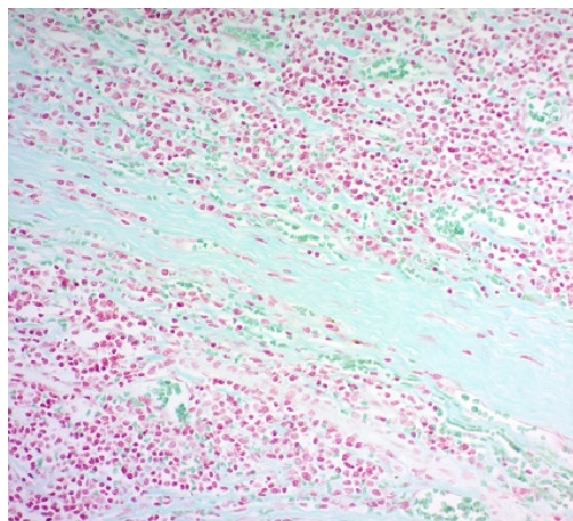
(Add components in the order listed)

- |       |                              |
|-------|------------------------------|
| 30 ml | Distilled Water              |
| 9 ml  | Twort's Neutral Red (Part A) |
| 2 ml  | Twort's Fast Green (Part B)  |

### Procedure:

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Stain slide in Working Twort's Counterstain for 5-10 minutes. Use once and discard.
3. Rinse quickly in distilled water.

**Note:** for greatest retention of stain, air dry, clear and mount at this point. Alcohol and decolorizer solutions can quickly remove stains.



Human Spleen stained with Twort's Counterstain Kit

4. Dip once in absolute alcohol.
5. Decolorize quickly and carefully in Acetic Alcohol Solution.
6. Dip two times in absolute alcohol.
7. Clear, and mount in synthetic resin.

### References

1. Puchtler H., Waldrop F.S., Valentine L.S. Polarization microscopic studies of connective tissue stained with picro-sirius red FBA. Beitr Path. 1973; 150, pages 174-187.
2. Junqueira L.C.U., Bignolas G., Brentani R.R. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochemistry J. 1979, 11, pages 447-455.
3. Dolber, P.C., Spach, M.S. Picrosirius red staining of cardiac muscle following phosphomolybdic acid treatment. Stain Technology, January 1987, Volume 62(1): pages 23-26.
4. Whittaker, P., Kloner, R.A., Boughner, D.R., Pickering, J.G. Quantitative assessment of myocardial collagen with picrosirius red and circularly polarized light. Basic Research in Cardiology. 1994, Volume 89, Number 5, pages 397-410. DOI: 10.1007/BF00788278
5. Whittaker P. Polarized light microscopy in biomedical research. Microscopy and Analysis 1995; 44, pages 15-17.



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