

Twort's Gram Stain Set - Technical Memo

SET INCLUDES:

Solution A: Neutral Red Stain 1%, Alcoholic
Solution B: Fast Green Stain 1%, Alcoholic

Part 14034A

250 ml
100 ml

Part 14034B

500 ml
200 ml

Additionally Needed For Gram Stain, Hucker-Twort:

Gram, Multi-Tissue, Artificial Control Slides
Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort
Iodine, Weigert & Lugol, Aqueous
Xylene, ACS
Alcohol, Ethyl Denatured, 100%
Alcohol, Ethyl Denatured, 95%
Acetone, ACS

Part 4256 or Gram+ & Gram- Bacteria, Artificial Control Slides Part 4255
Part 10422
Part 12092
Part 1445
Part 10841
Part 10842
Part 10014

For storage requirements and expiration date refer to individual product labels.

APPLICATION:

Newcomer Supply Twort's Gram Stain Set provides stain solutions for the Gram Stain, Hucker-Twort, a rapid and simple procedure that stains gram-positive and gram-negative bacteria without the use of picric acid. The Neutral Red Stain combined with a Fast Green counterstain provides clear detection of any red gram-negative bacteria present against a green background.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)

Technique: Paraffin sections cut at 4 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Sets are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure. Some solutions in the set may contain extra volumes.

PRESTAINING PREPARATION:

- If necessary, heat dry tissue sections/slides in oven.
- Filter Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort (10422) with high quality filter paper.

STAINING PROCEDURE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - See Procedure Note #1.
- Stain in freshly filtered Crystal Violet-Oxalate Stain, Alcoholic, Hucker-Twort (Step #2) for 30 seconds.
- Rinse quickly in distilled water.
- Mordant in Iodine, Weigert & Lugol, Aqueous (12092) for 20 seconds.
- Rinse quickly in distilled water.
- Decolorize individually with Acetone, ACS (10014); 2 quick dips.
 - Or until majority of purple stain is removed and tissue remains light gray.
- Rinse quickly in distilled water.
- Prepare fresh Twort Stain; combine and mix well.
 - Solution A: Neutral Red Stain 1%, Alcoholic 9 ml
 - Solution B: Fast Green Stain 1%, Alcoholic 3 ml
 - Distilled Water 30 ml
 - Use within 30 minutes.
- Stain in fresh Twort Stain for 2 minutes.

- Rinse quickly in distilled water and carefully blot dry.
- Agitate slides quickly in clean Acetone, ACS to dehydrate (do not use any alcohols).
 - See Procedure Notes #2 and #3.
- Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

| | |
|-------------------------------|-----------------|
| Gram-positive bacteria | Dark blue |
| Gram-negative bacteria | Red |
| Cytoplasm and red blood cells | Shades of green |
| Nuclei | Red |

PROCEDURE NOTES:

- Drain slides after each step to prevent solution carry over.
- If needed, add extra dips in acetone to further differentiate and dehydrate sections.
 - Check microscopically to avoid over-differentiation.
- The use of alcohol will remove Neutral Red staining.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Bancroft, John D., and Alan Stevens. *Theory and Practice of Histological Techniques*. 3rd ed. Edinburgh: Churchill Livingstone, 1990. 290-292.
- Culling, C.F.A. *Handbook of Histopathological and Histochemical Techniques*. 3rd ed. London: Butterworth, 1974. 393-395.
- Twort, F.W., "An Improved Neutral Red, Light Green Double Staining for Animal Parasites, Microorganisms and Tissues". *Journal of State Medicine* 32. (1924). 351.
- Modifications developed by Newcomer Supply Laboratory.