

Technical Data Sheet

Modified Hiraoka Grid Staining Kit

Introduction

The Modified Hiraoka Grid Staining Kit is based on the classic publication by Hiraoka (1972) and modified to line the inner tray wells with disposable inert sheets (Seifert 2017). The kit can handle up to 20 TEM grids at a time for heavy metal staining of biological samples.

Unique features of this kit include, but are not limited to:

- Reduced risk of mechanical damage
- Simple alpha-numeric system for easy grid identification and location on grid support plate
- Controlled time and stain exposure of all TEM grids
- Minimal to non-existent stain precipitate using ParafilmR lined trays and no need to clean the tray after each use.

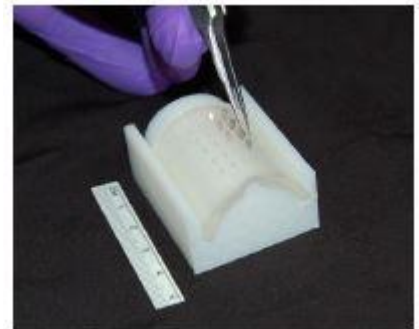


NOTE: The plastics that are used in this kit are not resistant to alcohols and acids. The staining trays are specifically designed for aqueous solutions.

NOTE: DO NOT USE stains that are alcohol-based as they will cause substantial damage to the materials of both ParafilmR tray liners and trays.

Examples of Stain Use

- Aqueous uranyl acetate (0.5-2%)
- Non-radioactive uranyl acetate replacement solutions
- Lead stain (e.g. Reynold's, Sato's)
- NOTE: Use pre-boiled and cooled reverse osmosis distilled water (distilled water) for all rinses.
- Use 0.2 μ m filtered stain solutions immediately prior to use in trays



Volume of Stains

- 1-10 grids: 2ml, using Staining tray space insert in tray well to decrease volume
- 11-20 grids: 5ml, full tray volume

Instructions

1. Place Grid support plate into the Modified Hiraoka plate holder and stretch at an arc and clip into corners with slits facing upward to receive individual TEM grids.



2. Load TEM grids into slits of Grid support plate with fine forceps at identified locations then slowly unclip from the Modified Hiraoka plate holder.

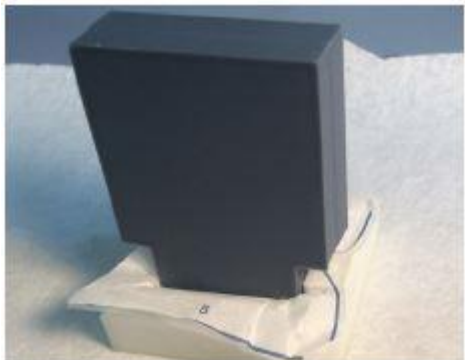
For ParafilmR lining of full tray well surface

- Place ParafilmR sheet over Modified Hiraoka staining tray well with lining sheet upward.
- Use ParafilmR well mold to insert ParafilmR sheet into tray well surface and outer edges.
- Use ParafilmR well mold to crease edges ParafilmR around edges of tray. Slowly peel away ParafilmR lining sheet from edge of tray to remove. After removal, use lining sheet to press edges of ParafilmR into edges of tray for adhesion into the tray well.



For lining half of tray well surface to minimize volume (2ml)

- Insert Staining tray space insert into Modified Hiraoka staining tray well and place ParafilmR sheet on top with lining sheet upward.
- Use the protruding end of ParafilmR well mold to insert ParafilmR sheet into tray well surface and outer edges.
- Use ParafilmR well mold to crease edges ParafilmR around edges of tray. Slowly peel away ParafilmR lining sheet from edge to remove. After removal, use lining sheet to press edges of ParafilmR into edges of tray for adhesion into the tray well.



3. Fill ParafilmR lined trays with stain solutions and distilled water liquids. There should be a positive meniscus of liquid in each tray.
4. Use laboratory established TEM grid staining procedure with a minimum of 4 distilled water rinses after each stain (e.g. uranyl acetate and lead stain). Place the grid support plate with the mounted grids into each filled tray to totally submerge grids into the stain solutions and subsequent distilled water rinses at an angle to prevent air bubbles. There may be a residual amount of liquid spillage that should flow into the outer trough of each tray.
5. After staining run completion, dispose all used solutions and ParafilmR tray liners according to local, state, and federal regulations

References

Hiraoka JI. **A holder for mass treatment of grids, adapted especially to electron staining and autoradiography.** Stain Technology 1972; 47:297–301.

Seifert, P. **Modified Hiraoka TEM grid staining apparatus and technique using 3D printed materials and gadolinium triacetate tetrahydrate, a nonradioactive uranyl acetate substitute.** Journal of Histotechnology. 2017. 40 (4):130–135.

