Preparing Macromolecular Specimens for TEM: Negative Staining of DNA-RecA Complex

mPrep™ System

Applications Note #503

Introduction

Negative staining is a common method to prepare macromolecular and particulate specimens such as protein complexes, DNA/RNA oligonucleotides, nanoparticles, and viruses for transmission electron microscopy (TEM). The typical lab procedure uses manual droplet grid handling methods to apply a suspension or solution of the specimen to Formvar®, carbon or other filmed TEM grids. After the specimen adsorbs, the filmed grids are then carefully rinsed, negatively stained with uranyl acetate or other heavy metal, and rinsed again. This method, like all droplet staining methods, requires considerable manual grid handling, and can be especially tedious if more than a few grids are being prepared. As with all manual grid handling, it is easy to drop, lose, mix-up and otherwise damage the grid, and especially all too easy to tear the supporting film when handling filmed grids.

Herein we demonstrate how negative staining may be performed easily with multiple grids with a much lower risk of damage to the grid or to the fragile thin film. Instead of considerable manual handling of grids with forceps, the filmed grids are inserted into mPrep/g™ capsules where they remain safely and securely encapsulated and labeled. The mPrep/g capsules are then connected to a common laboratory pipettor for all fluid preparative procedures, from applying the specimen solution, through all stain and rinse steps (Figure 1).

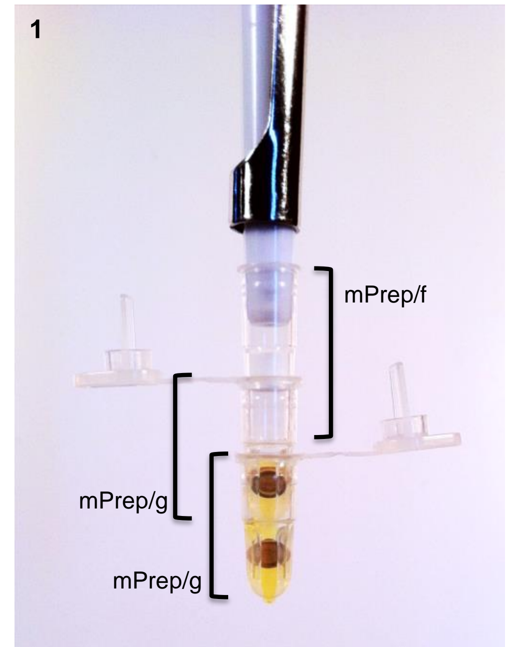


Figure 1: Two mPrep/g capsules with two grids inserted into each capsule are mounted in series onto a pipettor via an mPrep/f filter coupler. The pipettor volume is set to 80 µl to fill both mPrep/g capsules with stain.

Sample Preparation

DNA RecA protein complex from Escherichia coli was prepared by the method described in Lusetti et al.[1] Conventional 400 mesh copper grids were prepared with Alcian blue activated carbon films, also using the methods described in Lusetti et. al.[1] The filmed grids were then inserted into mPrep/g capsules and stored until use.

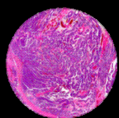
Staining Protocol

The mPrep/g capsules with carbon-filmed TEM grids were mounted onto a Gilson Pipetman® (Figure 1). The aspiration and dispense volume was set to 80 µl to fill the two stacked mPrep/g capsules:

1. DNA-RecA complex solution was aspirated into the mPrep/g capsules and held for 3 minutes to allow the macromolecular complex to adsorb to the carbon film. After 3 minutes the DNA-RecA solution was dispensed to waste.

---

[1] Lusetti SL, Wood EA, Fleming CD, Modica MJ, Korth J, Abbot L, Dwyer DW, Roca AI, Inman RB, Cox MM (2003) C-terminal Deletions of the Escherichia coli RecA Protein: Characterization of *In Vivo* and *In Vitro* effects. J Biol Chem, **278**, 16372-16380.



MICROSCOPY™ INNOVATIONS LLC
213 Air Park Rd Suite 101
Marshfield WI 54449-8626
888.302.3925 or 715.384.3292
info@microscopyinnovations.com

© 2014 Microscopy Innovations, LLC

2. The grids were then rinsed twice with distilled deionized water, with the water held in the capsules for about 1 minute and then dispensed to waste.
3. Aqueous freshly filtered 2% uranyl acetate was then aspirated into the mPrep/g capsules, held for 30 seconds, and then dispensed to waste.
4. The grids were then rinsed twice with distilled deionized water. Capsule ends were then blotted and kept open to allow the grids to fully dry.
5. Prior to TEM imaging, the grids were removed from the mPrep/g capsules and rotary shadowed with platinum using standard methods.

Transmission Electron Microscopy

The prepared grids were imaged with an FEI Tecnai™ T12 at 80 KeV, over magnifications form 15,000 to 25,000 X, with images recorded to an FEI Ultrascan™ Camera (Figure 2).

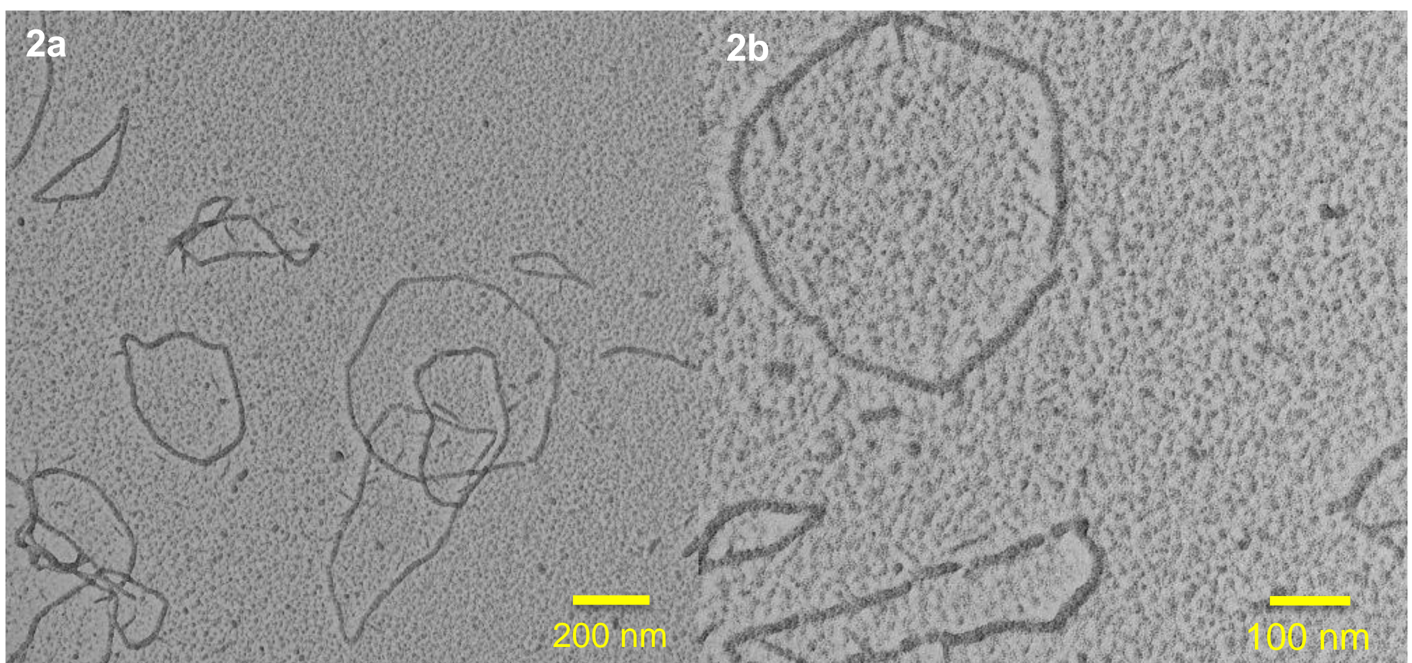


Figure 2: Electron micrographs of DNA-RecA complex at lower (a) and higher resolution (b).

Results

The overall quality of negative staining was comparable to conventional droplet staining, while also providing these advantages:

- Much less grid handling to reduce the potential for grid loss, damage, or mix-up.
- Since grids in the mPrep/g capsules are held parallel to the fluid flow direction, the forces on the carbon film were much less than with droplet methods, thus further reducing the potential for damage to the supporting film.
- Controlled and uniform stain exposure and consistent rinsing in sealed capsules provides reliable results.
- Multiple grids are prepared at once, saving time and providing identical process timing for all grids.
- Method can be extended to prepare even more grids: Up to 8 grids can be prepared simultaneously with the same procedure by stacking up to 4 mPrep/g capsules onto a

single channel pipettor (Figure 1). Or, even more grids can be prepared using a multichannel pipettor (see AN502).
- Reagent consumption of 20 µl per grid reduces purchase and disposal costs.
- Reagents stain grids in enclosed capsules thus reducing exposure to toxic chemical stains, enhancing user safety and providing universal hazards compliance.
- Industry-standard sized mPrep capsules fit on a wide range of manual or automated liquid handlers to provide throughput from one grid to thousands of grids per day.

Conclusions

Using mPrep/g capsules and pipettor processing provides staining that is at least comparable to conventional droplet methods, but with greater ease and reproducibility and much less risk of loss, damage or mix-up. In this example, there were seven (7) applied fluids: DNA-RecA specimen, two (2) water rinses, uranyl acetate stain, and two (2) final rinses. Even with only seven (7) fluids this provided a significant reduction in grid handling compared to manual droplet preparation. For procedures where there are more liquid steps, such as those where the filmed substrate is first treated to enhance adsorption or induce cross-linking, and/or where immuno or other labels are used, the ease and reproducibility of sample preparation would be further increased compared to droplet methods.

Unlike droplet staining methods where many users apply the specimen and stain to only one side (or face) of the grid, in the mPrep/g method described herein, the specimen and stain were applied to both sides. This produces no deleterious effects and provides at least two advantages: Firstly, this eliminates the potential to accidently rotary shadow the side of the grid without the adsorbed specimen (in the present study where rotary shadowing was used), and secondly this doubles the surface for adsorption. The latter can be particularly beneficial when working with low specimen concentrations. Finally, since the mPrep/g capsule is sealed, it is easy to greatly extend the adsorption time for specimens present in low concentration or for those that adsorb slowly.

Acknowledgement

We gratefully acknowledge Dr. Michael M Cox, Dr. Sindhu Chitteni Pattu, and Mr. Jay Campbell (Department of Biochemistry, University of Wisconsin-Madison) for performing this study and for providing permission to share this work.

Ordering Information

Product #	Item Description/Catalog Information
G1600	16 mPrep/g capsules & 16 label sets in capsule/grid storage box
F1601	16 mPrep/f standard pore filter couplers in capsule/grid storage box
KIT_xxx	Starter kits with mPrep capsules and accessories including Gilson Pipetman® (various custom kits available — please inquire)

AN503 rev1