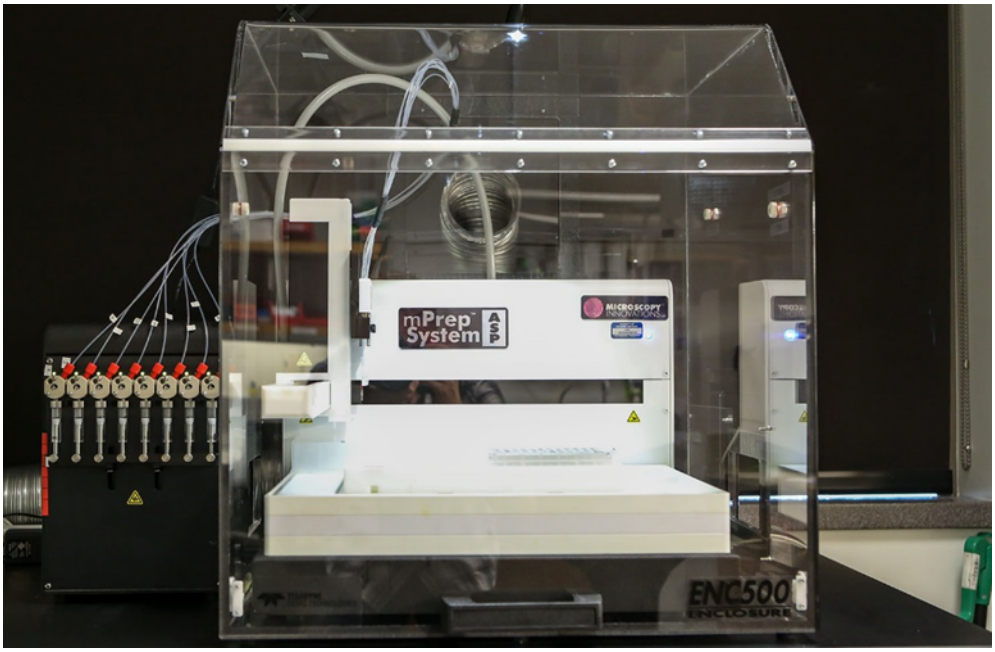
Automated Immunogold Labeling of TEM Grids

mPrep™ System

Technical Note

Immunogold labeling (IGL) was introduced in the 1970s to enable electron microscopists to localize the constituents of a cell by attaching (electron dense) antibody-bound colloidal gold particles to antigens on specific cellular components. Here we demonstrate use of the Microscopy Innovations mPrep™ System ASP-1000 Automated Specimen Processor for this application.

Scientists at the University of Wisconsin Medical School Electron Microscope Facility used the mPrep™ System ASP-1000 Automated Specimen Processor to perform unattended, fully-automated IGL grid preparation using an Aurion™ blocking buffer and protocol with excellent results.



ASP-1000 Automated Specimen Processor

Thin sections of resin-embedded, nematode samples (*C. elegans*) were placed on TEM grids, which were then inserted into mPrep/g™ capsules. The capsules were mounted on the ASP-1000 and processed unattended to accomplish IGL in 7.5 hours. The following Aurion protocol was utilized:

Reagent	Incubation Time (minutes)	Rinsing Time (minutes)	Number of Rinsing Steps
Glycine	15	n/a	n/a
Aurion Blocking Solution	15	5	3
Primary Antibody	120	5	6
Secondary Antibody (gold conjugate)	120	5	9
Glutaraldehyde	5	5	9

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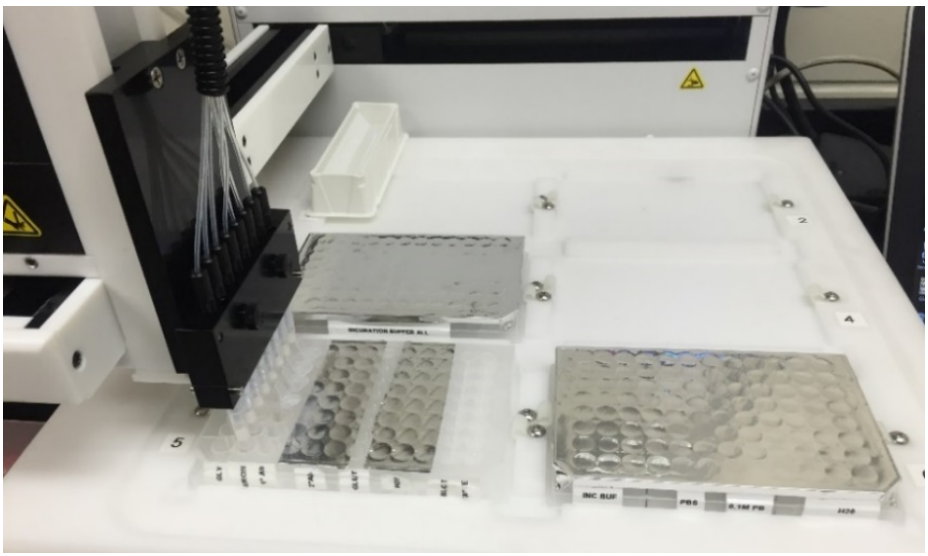

Reagents on the ASP-1000 deck were stored in 96-well sealed microplates.

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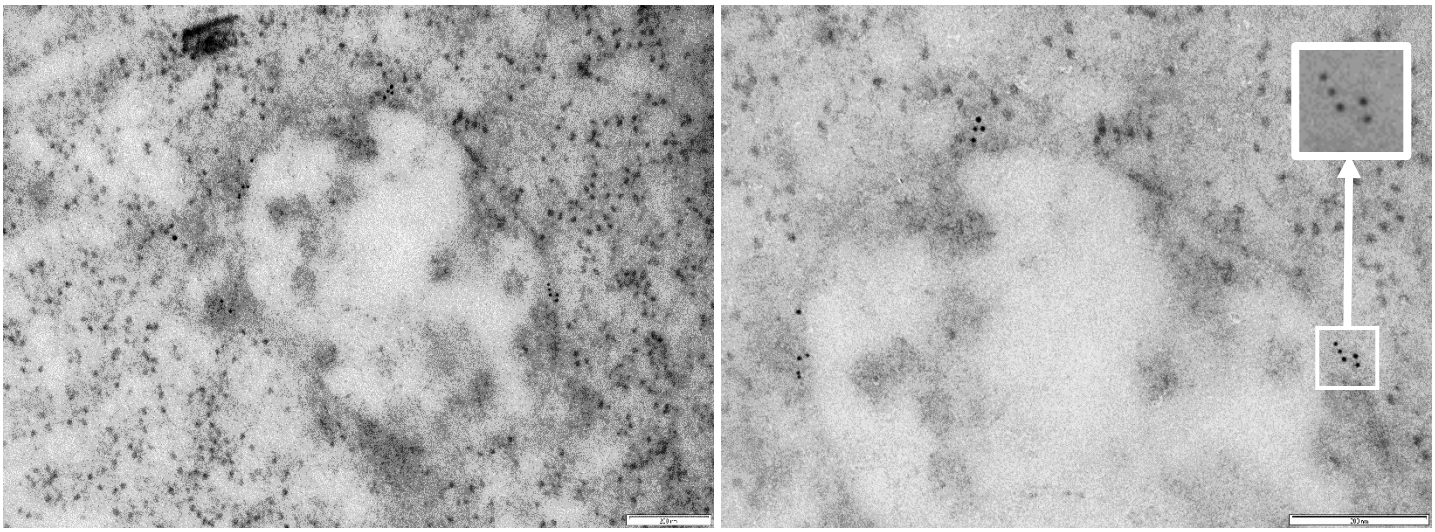
Rinsing reagents were pre-dispensed into 96-well polypropylene microplates and sealed with aluminum film. Glycine, glutaraldehyde and antibodies (primary and secondary gold conjugate) were added to the microplate immediately prior to starting the pre-programmed protocol. Samples were automatically agitated by dispensing and immediately re-aspirating 40 µL every 10 minutes during antibody incubations and every 15 seconds during rinsing steps. Upon protocol completion, transmission electron microscopy (TEM) was performed at the UW Medical School EM Facility.



A component of the endosomal sorting complex required for transport (ESCRT) machinery localizes to the limiting membrane of a multivesicular endosome in a *C. elegans* 1-cell stage embryo, as revealed by immunogold labeling (6 nm Au particles). Scale bars = 200 nm.

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