



Twin Cities Campus

Imaging Center

College of Biological Science

Mark A. Sanders, Program Director

msanders@umn.edu

www.cbs.umn.edu/ic

23-37 SnyderHall

1445 Gortner Avenue

St. Paul, MN 55108-5754

Phone: 612-624-3454

Fax: 612-624-1799

Immunolabelling of LR White/Gold Sections

1. Prepare clean labeling surface by laying down a long piece of parafilm on a wet bench and pressing it down to make it adhere tightly.
2. Quench unreacted aldehyde groups by placing grids on drops of 0.1M NH₄Cl in PBS for 10 minutes @ RT.
3. Block non-specific binding sites by transferring grids on drops of 1% fish skin gelatin in PBS, for 20 minutes @ RT.
4. Prepare moist chamber using large Petri dish and pieces of filter paper soaked with water. Incubate grids on 10ul drops of primary antibody diluted in PBS/1% FSG for 30 min @ RT.
5. Wash 4 x in PBS (5 minutes total).
6. Label grids with secondary antibody (if necessary) for 30 minutes @ RT.
7. Wash 4 x in PBS (5 minutes total).
8. Label grids with Protein A Gold for 30 min @ RT.
9. Wash 4 x in PBS (5 minutes total).
10. Fix in 1% glutaraldehyde in PBS for 5 min.
11. Wash grids 5 x with distilled water. Air dry.
12. Stain grids with 2% uranyl acetate and lead citrate.