Immunogold labeling of bacterial cells for transmission electron microscopy (TEM)

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Abstract

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Materials

- 1X PBS (Phosphate-buffered saline) by Contributed by users
- 70% Ethanol by Contributed by users

Paraformaldehyde Aqueous Solution -16% 15700 by Electron Microscopy Sciences

Anti-Rabbit IgG (whole molecule)–Gold antibody produced in goat Sigma 0162787 by Sigma

- 5% BSA in PBST by Contributed by users
- Formvar-coated 300-mesh electron microscopy grids by Contributed by users

Protocol

Step 1.
Cut a strip of Parafilm (20-30 cm long), place flat on bench.

Step 2.
Prepare one 1-mL tube each of 70% ethanol and mqH20 for washing tweezers between transfers.

Step 3.
Harvest cells by flooding an overnight plate with 5 mL nutrient broth or buffer, using a spreader to dislodge cells, pipetting into a conical flask and mixing gently by inversion (avoid pipetting up and down, vortexing and centrifuging to preserve flagella).

Step 4.
Set cells to an OD600 of 1-3 in NZCYM broth (1-mL volume), place on ice until use.

Step 5.
Pipette 100 uL cells onto parafilm, use tweezers to float Formvar-coated copper (300 mesh) grid atop droplet, Formvar-side-down (should do this in duplicate, ie. 2 grids per drop), incubate 45 min at RT (between transferring grids, particularly between transfer of grids containing different samples, twirl tweezers 2-3 seconds in EtOH, then in H20, then wipe dry with a fresh Kimwipe)

Step 6.
Wash grids 1x by transferring to a 100-uL drop of PBS, incubating 3-5 min.

Step 7.

Incubate 20 min in 2.5% paraformaldehyde/PBS to kill cells and fix them to grids.

Step 8.
Wash 3x in PBS (100 uL drops, 3-5 min each).

Step 9.
Block in 5% BSA/PBST (100 uL, 35 min).

Step 10.
*If doing Far Western blot, ie. testing protein binding to cells, incubate in desired protein diluted 1/25 in blocking solution, 45 min, 100 uL drop

Step 11.
Wash 3x in blocking solution (100 uL drops, 3-5 min each).

Step 12.
Incubate in primary antibody (anti-Gp047, rabbit) at 1/50 (diluted in blocking solution), 45 min, 100 uL.

Step 13.
Wash 3x in blocking solution (100 uL drops, 3-5 min each).

Step 14.
Incubate in secondary antibody (goat anti-rabbit IgG-gold) at 1/50 (diluted in blocking solution), 45 min, 100 uL.

Step 15.
Wash 3x in blocking solution (100 uL drops, 3-5 min each).

Step 16.
Wash 3x in PBS (100 uL drops, 3-5 min each).

Step 17.
Wash 3x in water (100 uL drops, 3-5 min each) to remove salts, which can form crystals under EM.

Step 18.
Dry completely on Whatman filter paper in a Petri dish (at least 20 min, preferably overnight). OK to store grids in this way at RT for several days prior to TEM, but ideally visualize within 1 day.

Step 19.
Analyze grids via TEM.