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Preparation performed by investigator prior to sample submission to the Core Facility

Primary Fixation of Suspensions (e.g., cells, bacteria, virus)

Cells

1. Cell culture media is removed from culture plate and washed 3 times with 0.1M PIPES or PBS buffer (pH 7.2 - 7.4).
2. Fixation of cells with glutaraldehyde 2.5 % (EM grade) freshly prepared in 0.1 M PIPES or PBS (pH 7.2 - 7.4) buffer for 30 min to 1 hr. at RT.
3. Rinse 3 x 5 min with PIPES or PBS as before.
4. Scrape the cells from culture plate in buffer, spin down and pellet at 1000 g for 5 min in 1.7 ml Eppendorf tube identified with permanent label. Please make sure to obtain a visible pellet (~1 million cells).
5. Submit fixed samples to the Core Facility in PBS buffer and identify with permanent labels.

Bacteria, Virus

1. Centrifuge the suspension at an appropriate speed that will yield a pellet.
2. Remove the supernatant and add glutaraldehyde 2.5 % (EM grade, freshly prepared in 0.1 M PIPES or PBS buffer, pH 7.2 - 7.4) down the wall of the Eppendorf tube avoiding disintegration of the pellet. Fix for 30 min to 1 hr at RT.
3. Centrifuge, remove the fixative and add PIPES or PBS as before for 10 min.
4. If the pellet resuspends it can be recentrifuged.
5. Submit fixed samples to the Core Facility in PBS buffer and identify with permanent labels.