QUANTOMIX™			
Protocol for Sample Preparation (QX-102)			
Doc. No.: QXP005	V 1.0	**Title: Osmium Tetroxide Staining for Cells**	
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Introduction

Osmium Tetroxide is traditionally used in electron microscopy both as a fixative and a heavy metal stain. Osmium Tetroxide is a good fixative and excellent stain for lipids in membranous structures and vesicles. The most prominent staining in adherent human cells (HeLa) is seen on lipid droplets. Some intracellular structures are also visualized. Visualized cellular structures depend on the fixation protocols; in Glutaraldehyde fixation nucleoli are visible, but overall nuclear staining is weak. In Paraformaldehyde fixation nuclear staining becomes more prominent, but some intracellular structures are lost. As a first choice, fixing with a combination of Glutaraldehyde and Paraformaldehyde is recommended.

WARNING

Since OsO_4 is toxic and volatile, all work should be performed in a fume hood using gloves and protective clothing. Handling and waste disposal should be done according to the guidelines of the local authorities.

The reagents required

- 4% OsO_4 (for example, Fluka Cat. No. 75632)
- 2% Paraformaldehyde/0.1% Glutaraldehyde in PBS
- Double distilled water
- PBS

Procedure

1. Wash the sample four times with PBS.
2. Fix with 2% Paraformaldehyde, 0.1% Glutaraldehyde in PBS for 30 minutes.
3. Wash four times with PBS.
4. Wash four times with double distilled water.
5. Prepare 0.1% OsO_4 solution by diluting the 4% stock solution in double distilled water.
6. Incubate the sample with 0.1% OsO_4 for 30 minutes.

NOTE

The optimal incubation time and dilution may vary between samples and should be experimentally determined.

7. Wash four times with double distilled water.

NOTE

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