QUANTOMIX™			
Protocol for Sample Preparation (QX-102)			
Doc. No.: QXP002	V 1.0	**Title: Bacteria OsO_4 Staining**	
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Introduction

The QX-102 capsules can be used for imaging microorganisms grown in suspension. Since these organisms usually do not attach directly onto the capsule membrane, coating of the membrane is required. Preferred attachment protocols make use of Poly-L-Lysine or Gelatin coating. Bacteria is attached to the coated capsule membrane by incubating or by centrifuging. The suspension of bacteria can be maintained in a culture medium or buffer and may be fixed to the membrane before application. The dilution factor of the suspension depends on the type of organism and culture used and should be determined experimentally.

➢ The reagents required

- Bacteria suspension at the appropriate dilution (for example, for E.coli, 1:100 dilution of an over-night culture renders the appropriate number of Bacteria in a capsule).
- 0.1% Poly-L-Lysine solution
- 2% Paraformaldehyde/0.1% Glutaraldehyde in PBS
- 4% OsO_4 (for example, Fluka Cat. No. 75632)
- PBS
- Double distilled water.

⚠

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.

Procedure

➢ Capsule -membrane Coating

1. Apply 15 µl of 0.1% Poly-L-lysine solution to the liquid dish and incubate for one hour at room temperature.
2. Remove the solution and wash three times with double distilled water.
3. Remove the water and let the liquid dish dry over-night in the air.

Sample Preparation

1. Apply 15 µl of the Bacteria suspension into the liquid dish and incubate for one hour at room temperature, or centrifuge at 3000rpm for 5 minutes in a centrifuge equipped with 96 well plate holder.
2. Wash the sample three times with PBS.
3. Fix with 2% Paraformaldehyde/0.1% Glutaraldehyde/PBS for 30 minutes.
4. Wash the sample four times with PBS.
5. Wash the sample four times with double distilled water.
6. Prepare 0.1% OsO_4 solution by diluting the 4% stock solution in double distilled water.
7. Incubate the sample with 0.1% OsO_4 for 30 minutes.
8. Wash four times with double distilled water.
9. Prior to imaging, exchange the liquid in the dish to 15 µl QX-102 Imaging Buffer.

NOTE

Protocols provided by QuantomiX are based on work conducted at QuantomiX laboratories. They are given as a starting point which will facilitate the user's first steps in acquiring the desired imaging results. It is the user's responsibility to determine the suitability of any protocol published by Quantomix to their applications. Users may find it necessary to modify protocols in order to obtain the information required for their study.