# Bacterial Fixation Protocol

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| **Written by**: | **Date:** 15th May 2019 |
| **Approved by**: Dr. Susu Zughaier | **Protocol Number**: P003 |

**Background:**
Fixation of bacteria is done to preserve the 3D structure of bacteria. Using formalin fixed bacteria in experiments allows the study of interactions between cell surface receptors in bacteria and human cells. Formaldehyde is a chemical commonly used for fixation. It fixes cells by cross-linking proteins, mainly the residues of the amino acid lysine, which terminates all biochemical reactions within the cell.
Formalin is a concentrated solution of Formaldehyde. 100% formalin = 37-40% formaldehyde.
Optical density is the ability of a medium to absorb/block the transmission of light passing through it. Optical density measured at a wavelength of light equivalent to 600 nm is usually used to estimate the amount of bacteria present in a solution.

**Reagents:** (these amount are to prepare 50 mL of 4% formaldehyde solution)

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| 40% Formaldehyde | 10 mL |
| PBS | 40 mL |
| Nutrient Agar Plates | As Needed |

**Materials:**
50 mL conical tube
Sterile loops or cotton swabs
25 mL pipettes
Pipette Gun
Cuvette (for spectrophotometer)

**Procedure:**

**a) Preparation of 4% formaldehyde (10% formalin)**

1. If you have 4% formaldehyde read skip this step to section b.
2. To prepare 4% formaldehyde solution you would need to perform a 1:10 dilution for formaldehyde stock of 40%. Hence, for 50 mL, 10 mL of stock is required and 40 mL of PBS (diluent)
3. Under the fumehood, transfer 10 mL from stock (40% formaldehyde) to the conical tube.
4. Under the biosafety cabinet aspirate 40 mL of sterile PBS to the conical tube.
5. Mix the solution by gently moving the tube forward and backward.

**b) Bacterial culture and fixation:**

1. Retrieve nutrient agar plates from the fridge and allow them to cool to room temperature. Label your nutrient agar plates as you go with: Student name, date, type of bacteria.
2. If using bacterial stock from -80 $^{o}$C then: take the vial to the biosafety cabinet, open the vial, using a sterile loop scrape the upper layer of frozen bacteria to get some of

it on the loop. Start streaking your plate using 4 quadrant streaking method. Immediately, take back the vial to -80 $^{o}$C so that the broth doesn't thaw.
If using bacterial stock from previous plating then: select an isolated colony and touch it with the tip of the sterile loop, start streaking the plate using 4 quadrant streaking method.

3. Incubate the nutrient agar plate at 37 $^{o}$C overnight (18-24 hours).
4. Next day, check nutrient agar plate for any abnormal or mixed growth. If all growth is pure, then using a loop or cotton swab harvest bacterial colonies from the agar plates into 50 mL conical tubes containing 3 mL 4% formaldehyde.
5. Make sure no clumps are visible, you could vortex to dissolve any bacterial clumps.
6. Allow the cells to rotate overnight at room temperature using electronic rotator.
7. Next day, centrifuge tube, discard supernatant, replace with PBS. Repeat this process 3 times to ensure the removal of formaldehyde as residual formaldehyde would affect your results. (NOTE: Discard formaldehyde waste in 50 mL conical tubes labeled "formalin waste" DO NOT DISCARD IN NORMAL WASTE).

**c) Adjusting optical density of bacteria:**

1. Adjust optical density of the spectrophotometer to OD 600.
2. Using sterile pipettes, take 900 uL of PBS and 100 uL of bacterial suspension prepared in section b. This gives 1:10 dilution of the bacteria (dilution is made to avoid wasting bacteria from stock, and also to avoid errors with spectrophotometer when OD is too high).
3. First, prepare a blank by adding PBS to a cuvette. Read the blank cuvette by pressing on "blank" button on spectrophotometer.
4. Read the optical density of the sample by pressing on "sample" button on spectrophotometer.
5. Once the result is shown, multiply the result by 10 to retrieve the actual optical density of your sample. (e.g. OD obtained is 0.1 , then 0.1*10 = 1). Using this information, you could dilute stock to desired optical density. (Hint: M1V1 = M2V2).

**Storage Conditions:**

| Reagent | Storage |
|---|---|
| 4% Formaldehyde | 4 - 25 $^{o}$C |
| Formalin Fixed Bacteria | 4 $^{o}$C |

**Hazards**:

| Reagent | Hazard |
|---|---|
| PBS | None |
| Nutrient agar | None |
| Formaldehyde  |  - Flammable liquid and vapor<br>  - Toxic if swallowed<br>- Toxic in contact with skin<br>  - Causes severe skin burns and eye damage<br>- May cause an allergic skin reaction<br>  - Toxic if inhaled<br>- May cause respiratory irritation<br>- May cause drowsiness or dizziness<br>- Suspected of causing genetic defects<br>- May cause cancer<br>- Causes damage to organs<br>- Causes damage to organs through prolonged or repeated exposure |