

# Biofilm Assay

## Protocol for Biofilm assay by Safranin using 96-well plates:

**Day 1:** Inoculate 5ml liquid medium with 5µl 1<sup>st</sup> Overnight culture, use disposable test tubes, and incubate at proper conditions overnight.

**Day 2:** Apply the second overnight culture into plates as your experimental designed pattern and perform the experiment as follow.

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## Experiment

- Apply **200µl** of diluted 2<sup>nd</sup> overnight culture (**1:100**) into each well and incubate at proper conditions overnight.
- Discard swimming (planktonic) bacteria and wash with 0.9% salt water (saline).
- Apply 200µl safranin (0.1%) per well to stain biofilm (sessile bacteria).
- Wait 5 Minutes, then discard unabsorbed safranin and wash once with 0.9% salt water (saline).
- Add 200 µl acetic acid (30%) into each well and wait 5 minutes
- Measure absorbance of samples at OD<sub>530nm</sub> (using BIO-TEK Synergy HT, USA)
- The plate with acetic acid solution should be packed in plastic bag, sealed and placed in the biological waste bin.

As option, let the solution evaporate and dried in a fume hood, then place it in the biological waste bin.



## Biofilm Assay

Measurement of bacterial biofilm

### Risk assessment is valid for

Determination of biofilm

### Analysis

The risk associated with this procedure will be minimized by following protocol.

List of Chemicals and Reagents			
Chemical/reagent	Link	Stock/ User Concentration	Special Waste Disposal?* Y/N
Acetic acid	<a href="#">MSDS</a>	98% / 30%	Collect the waste (solution 30%) in a disposable container or pack the applied plates for biofilm assay in a plastic bag before placing in Laboratory Waste Bin.
Safranin	<a href="#">MSDS</a>	0,1%	N

### Secure Job Analysis

Tasks	Unwanted Incidents	Precautions	Actions
Weighing of Safranin powder	Scattering of powder, inhalation and Eye irritation	Use nitrile gloves, lab coat and safety masks.	Drink plenty of water using eyewash system or eyewash spray head. Rinse eyes with water and Contact emergency if necessary.
Dilution of strong acetic acid 98%	spillage, splash and skin contact Burns on skin/eyes	Use nitrile gloves and lab coat. Dilution of concentrated acid should be done in fume hood	Cool skin with cold water. Rinse eyes with water using eyewash system or eyewash spray head. Contact emergency if necessary.
Usage of 30% acetic acid for destaining of bacterial biofilm	Spillage, splash and skin contact Burns on skin/eyes	Use nitrile gloves and lab coat. The solution should be applied in a fume hood or under a fume point.	Cool skin with cold water. Rinse eyes with water using eyewash system or eyewash spray head. Contact emergency if necessary.

## Applied bacterial strains in biofilm assay

Bacterial strain of biosafety level 2

### Risk assessment is valid for

Work with Bacterial strain of biosafety level 2

### Analysis

The risk associated with this procedure will be minimized by following protocol.

### List of Biological factors

Biological factors	Link	Stock/ User Concentration	Special Waste Disposal
Bacterial strain of biosafety level 2	<a href="#">Forskrift; kap.6 Vedlegg 2</a> <a href="#">WHO biosafety</a>	None	All contaminated materials should be placed in Biological Waste Bin. The bacterial culture waste should be autoclaved before placing in Biological Waste Bin.

### Secure Job Analysis

Tasks	Unwanted Incidents	Precautions	Actions
Usage of bacterial strains with biosafety level 2	Spill in laboratory area.  Contamination of instruments like centrifuge and incubator and so on.	Use lab gloves and lab coat.  Work should be done in biological safety cabinets.	Spray disinfectant solution EtOH 70% on contaminated area and wipe the area with wipe paper

# SynergyH1

Synergy H1 plate reader which is used for quantification of biofilm

## Risk assessment is valid for

Operation of Synergy H1

## Analysis

The risk associated with this procedure will be minimized by following protocol.

Instrument	Link	Stock/ User Concentration	Special Waste Disposal
Synergy H1 plate reader	Operator's <a href="#">Manual</a> Appendix A: Decontamination	None	None

## Secure Job Analysis

Tasks	Unwanted Incidents	Precautions	Actions
Loading of plate to read	Spill of chemicals and bacterial culture from plate	Use lab gloves and lab coat.  Cover plates with foil during reading	Spray disinfectant solution EtOH 70% on contaminated area and wipe off spill with wipe paper.

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