

# Crystal Violet Protocol for Biofilms

1. Remove media from biofilms and wash 1X in 1ml PBS
2. Let biofilms air dry 45min room temp
3. Add 1ml 0.4% Crystal Violet stain to each biofilm and let sit room temp  
45min
4. Remove Crystal Violet stain
5. Wash 4X with 3ml H<sub>2</sub>O gently to remove unbound stain
6. Add 2ml 100% EtOH to each biofilm and let sit 45 min room temp
7. Take out 3-4 100ul aliquots of EtOH, place in a 96 well plate, and read at  
595nm
  - a. Will need to use platereader in Proctor lab (currently called  
Pepperell lab) or Mansfield lab

## Important notes:

- Crystal Violet will stain almost anything it touches purple but is otherwise not dangerous
- Proctor lab closes at 5pm without special access
- Crystal Violet is not as variable or time sensitive as XTT, but I would not recommend trying to compare OD's read on different days. Only compare ODs read together in the same batch.