21 January 2022

# SOP# 28

## Chlorophyll a Determination Using Turner Trilogy

1. Pipette 50.0 uL of culture into a labelled 5.0 mL disposable tube glass culture tube (12 x 75 mm). **Record this volume.**
2. Add 2.0 mL of 3:2 solution of 90% acetone: DMSO to each tube containing your culture. **Record this volume.**
3. Prepare a blank tube with no culture.
4. Cover the tubes with aluminum foil to seal the tops and shield the sides from light and place in a dark cabinet for 30 minutes to 'extract'.
5. After 20 minutes, turn on the Turner Trilogy to warm up. Select the 'Chl-NA' method.
6. 5 minutes before your extraction is complete, place the solid-state standard in the cuvette holder of the Turner instrument, close the cover then press 'Measure Fluorescence'. Record the RFU value.
7. When the extraction time is finished (after 30 minutes), place each culture tube into the cuvette holder and take a fluorescence measurement. Record the RFU value.
8. This Turner instrument was calibrated on 19 January 2022 and found to be linear between RFU of 14 to 1345. RFU's outside of this range may yield erroneous results. **Redo your analysis.**

9. Record Sample ID, RFU and DF in your Chlorophyll a catalog which will be imported in R for ug $L^{-1}$ chl a calculation.

If your RFU is < 14, increase the volume of your culture from 50uL to an appropriate volume based on the RFU reading of your original sample and add enough 3:2 90% acetone:DMSO to bring the total volume to 2.050 mL. **Record your new volumes.**

If your RFU is > 1345, you need to dilute your sample extract. Determine your dilution factor (DF) by estimating what the new RFU will be. Aim for a mid-calibration range of 500-700 RFU. In this scenario a 1:1 dilution may be sufficient which is a dilution factor (DF) of 2.

Example: Original sample RFU = 1600. A DF of 2 would give RFU of ~800. Take 1.025 mL of your original sample and add 1.025mL of 3:2 90% acetone:DMSO. **Record this DF**.

R script uses slope = 7.0314 RFU/ug $L^{-1}$ and intercept of 0.9791