Tryptophan Fluorometer Calibration

Equipment Required (*Refer to Appendix):

- 1 Litre volumetric flask*
- 2 Litre volumetric flask
- 3 Litre container, non-reflective.
- 3 Litres of high grade, ultra-pure deionised water
- Small squeezy bottle (Approx. 250ml capacity) *
- L-Tryptophan powder
- Precision weighing scales and small weigh dish/boat*
- Magnetic stirrer and bean*
- High accuracy pipette for measuring 1ml of fluid*
- Digital Thermometer (accurate to 0.01 or 0.05, d.p, precision 0.1 d.p)
- Support Clamp (6kg capacity)

Ensure all fluid vessels are acid cleaned to remove traces of naturally occurring amino acids and all precautions taken to avoid the contamination of equipment during the process of making the stock solution and during the sensor calibration process.

Making Tryptophan Stock Solution

1. Accurately weigh out 0.2g of L-Tryptophan Powder.
2. Add the L-Tryptophan powder to an empty and clean 1Ltr Volumetric flask.

TIP: Use a squeezy bottle filled with Ultra-Pure Deionised Water to rinse the Tryptophan powder from the boat and inner surfaces of the flask neck, ensuring all powder enters the main bulb of the flask.

3. Accurately fill the flask to the 1Ltr mark with Ultra-Pure Deionised water.
4. Place the flask on the magnetic stirrer. Carefully add the stirring bean and switch on the stirrer to fully dissolve the powder in the water. This may take 20mins.

Add the bean after the flask has been filled with water in stage 3 to account for displacement.

This 1Ltr of stock solution has a Tryptophan concentration of 200mg/l or 200,000 ppb.

Where: 100mg (0.1g) L-Tryp powder in 1Ltr = 100mg/l or 100,000ppb

Therefore: 200mg (0.2g) L-Tryp powder in 1Ltr = 200mg/l or 200,000ppb

This Stock solution should be kept refrigerated and can expect to last 1 week

Making smaller volumes requires small amounts of powder which will significantly increase the risk of error in the final concentration value.

Cleaning and Preparation of the Proteus

New Units

If the Proteus is new, rinsing sensors with weak acid and deionised water will be sufficient before calibration.

Dirty Units

IMPORTANT NOTES.
DO NOT MANUALLY ROTATE THE WIPER ARM AT ANY POINT. THIS WILL PERMENENTLY DAMAGE THE SENSOR.
DO NOT USE A BRUSH ON, OR WIPE THE WINDOW/FACE OF OPTICAL SENSORS.
ENSURE THE SCREW CAP IS FITTED TO PROTECT THE 6 PIN CONNECTOR.

Rinse the sensors under running water.

Remove the wiper fitted to the Turbidity sensor by undoing the small grub screw with the Allen key provided. The rubber blade and nylon brush should be replaced if needed.

Ensure the bodies of ALL sensors are clean and all crevices between the sensors are free from dirt and deposits. Mild detergent and warm water can be used on sensors with a soft toothbrush and cotton buds. Rinsing under running water regularly. Once clean and loose debris rinsed off, the optical sensor windows can be lightly wiped with a clean cotton bud or optical cloth in one direction across the surface.

Using a squeezy bottle helps direct water between sensors to remove debris.

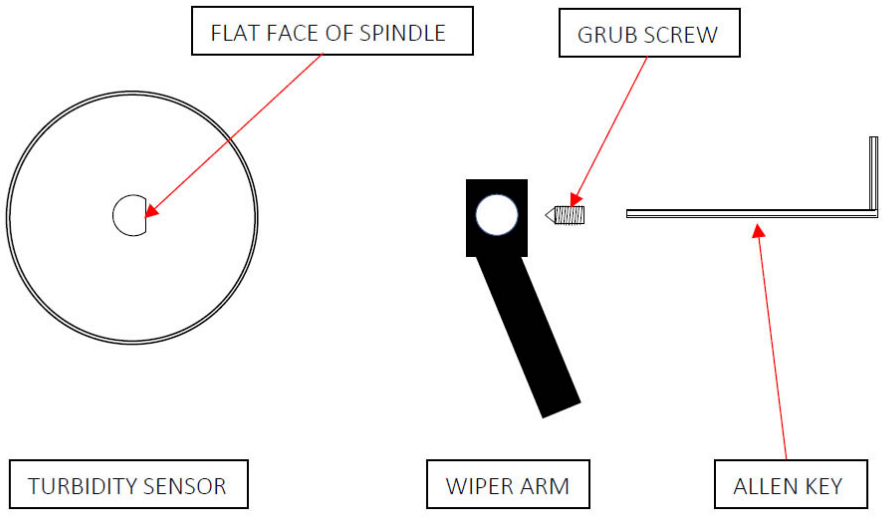
Only wipe sensor faces after the unit has been cleaned to avoid dirt scratching the surface.

Sensors can be soaked in a mild acid solution overnight (pH4). The screw on calibration cup should be used for this. Rinse the sensors under running water.

Ensure the Turbidity sensor wiper arm is refitted before calibration. Grub screw should mate with the flat face of spindle. Light pressure should be applied and the grub screw secured.

DO NOT over tighten and ensure the arm is level.

TURBIDTY SENSOR WIPER ARM



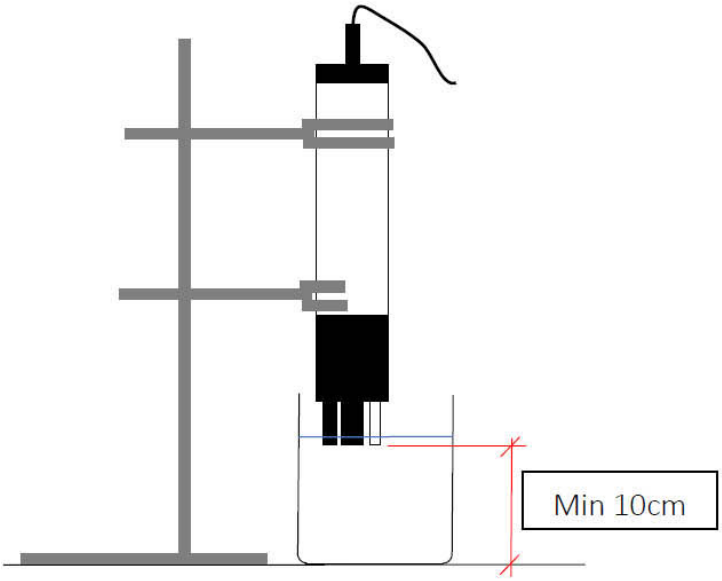
Calibrating the Tryptophan Sensor

Tryptophan calibration is a Two Point process:

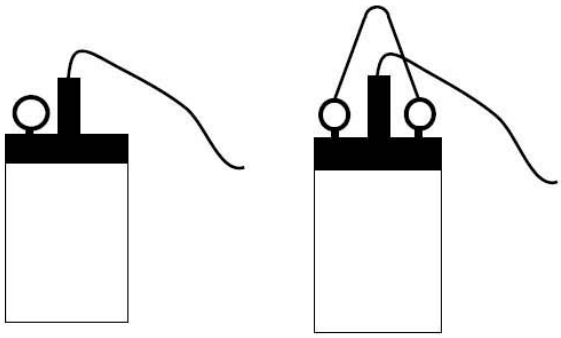
Ultra-Pure De-ionised water is used for First Calibration point with a Tryptophan value of 0ppb (Zero).

Adding 1ml of stock solution gives the water a Tryptophan value of 100ppb and is used for the Second Calibration point.

1. Accurately measure 2ltrs of Ultra-pure deionised water using the 2l volumetric flask and pour into a non-reflective container.
2. Using a clamp stand or similar method, secure and set up the Proteus with the sensors submerged in the deionised water to a depth of approximately 10-20mm, ensuring a minimum blanking distance of 10cm from the sensor windows to the bottom of the container.



Alternative hanging options using eye bolts and hanging wire provided. Ensure Proteus unit is well supported and hanging as straight as possible.



Locate the Tryptophan sensor and position it centrally to the container to create adequate edge distance.

After assuring the sensor is adequately submerged with correct blanking distance, all light must be blocked out.

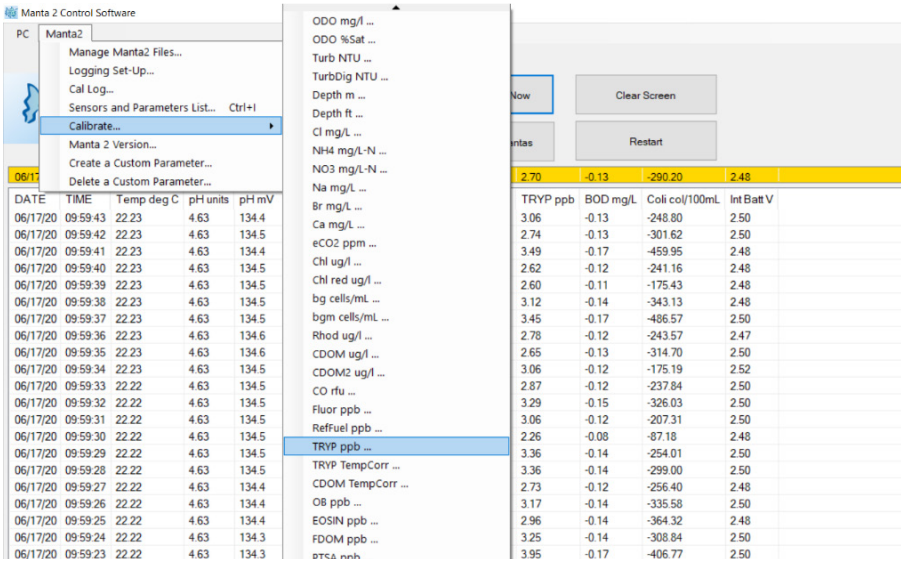
This can be done using a dark, non-reflective material (left). Avoid the use of plastic coverings as some plastics fluoresce. Ensure all lights are turned off.

Do not allow dust or fibres from any coverings to enter the water.

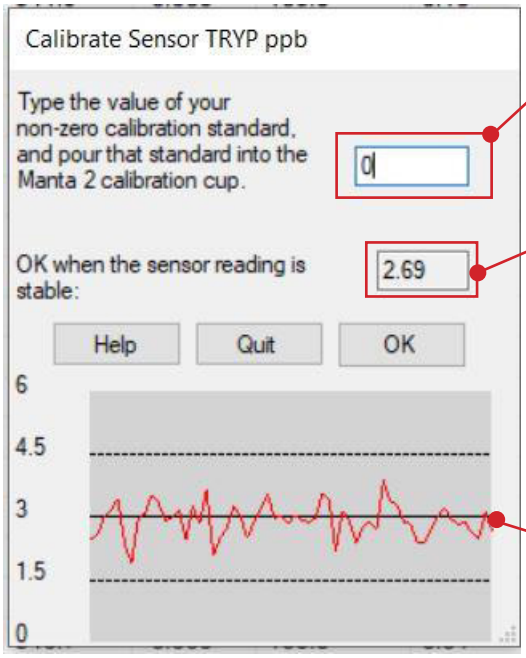
3. Connect the Proteus to the computer and open the software.
4. From the drop-down menu, select 'sensor and parameter list' and check [ ] the boxes for 'TRYP ppb' and 'TRYP TempCorr' so they both appear in the main scrolling page.

DATE	TIME	Temp deg C	pH units	pH mV	ORP mV	Stage m	HDO %Sat	Turb NTU	TRYP ppb	TRYP TempCorr	BOD mg/L	C
06/17/20	10:09:07	22.30	4.65	133.4	347.8	-0.006	105.8	0.12	89.34	98.16	9.46	-4
06/17/20	10:09:06	22.30	4.65	133.4	348.3	-0.005	105.8	0.09	90.62	99.57	9.80	-4
06/17/20	10:09:05	22.30	4.63	134.1	348.6	-0.005	105.8	0.12	91.90	100.96	10.16	-4

5. From the menu, select 'Calibrate' and 'Tryp ppb' from the drop-down list.



6. Enter '0' (zero) for the First calibration point.



Enter '0' (Zero) in this box. This is the Tryptophan value of the Ultra-Pure deionised water

This reading is the reading the sensor is currently reading and not representative of accuracy until calibration is completed. When stable select 'OK'. This reading will fluctuate a few points. Quality of water and process is critical for a good calibration.

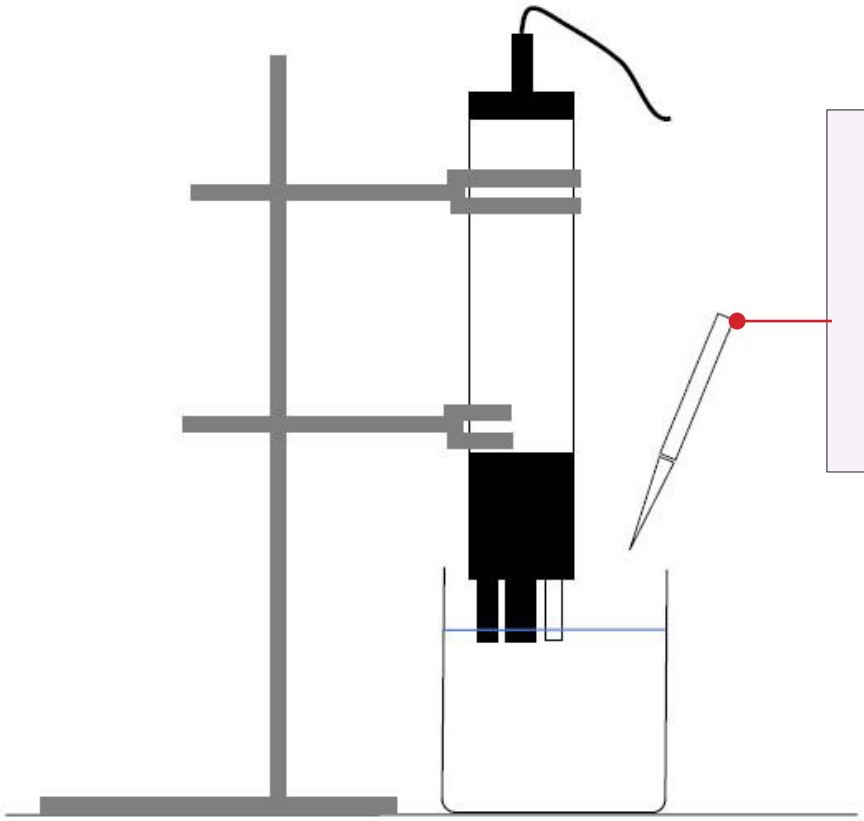
Allow line graph to travel full length of graph.

Select 'OK' when stable. A window will pop up briefly showing calibration successful, and a new window will open for the second calibration point.

DO NOT close any windows.

NOTE ABOUT SOFTWARE: The software will prompt for the first calibration point to be a non-zero value. The order in which a two-point calibration is conducted within the software is not critical. However, all sensors are initially calibrated in the order LOW to HIGH.

7. Using a pipette, add 1ml of stock solution (200,000ppb) to the 2 litres of deionised water. This creates a tryptophan solution with value of 100ppb. The container may need to be carefully agitated to mix.



Adding 1ml of the Tryptophan Stock solution creates a solution with a value of 100ppb. Gently swirl the beaker so the tryptophan is completely mixed into the DI water.

This solution is used for the second calibration point.

8. Use your thermometer to take the temperature of the solution as close to the Tryptophan fluorometer as possible. Do not allow the thermometer to come into contact with the sides or bottom of the container.
9. Use the excel spreadsheet calculator provided to obtain the Temperature Corrected value' of the water. Enter the value of the water (now 100ppb) and the temperature obtained in stage 5.

(1) These are our recommended buffer standard values. If you are using a different value standard, e.g. 500 ppb, make sure you adjust the below to reflect this.

(2) Enter the temperature of the solution in this column. Either use the temperature provided by the Proteus or a digital thermometer.

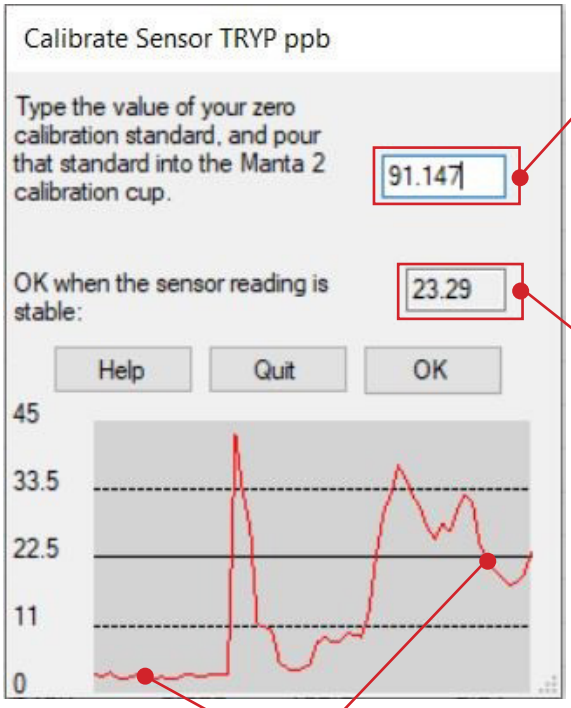
This is a fixed parameter. Do not change.

This is a fixed parameter. Do not change.

	Target Calibration Reading (Fixed)	Measured Temp (From Proteus or Thermometer)	Reference Temp (Fixed)	Temp Correction Coefficient (Fixed)
CDOM	100	9	20	-0.016
Tryp	100	17	20	-0.039
Chlorophyll-A	200	16	20	-0.014
		RAW Value (CDOM)	RAW Value (Tryp)	RAW Value (Chl)
		117.6	111.7	211.2

(3) Insert value from Row 13 as calibration standard value during the second calibration point.

10. Enter the Temperature Corrected value created by the calculator into the software box for the Second calibration point. (Ignoring software prompt for Zero Value as previously explained)



Temperature corrected value from calculator.

This reading is the reading the sensor is currently reading in the 100ppb solution and not representative of accuracy until calibration is completed. Note: the reading is higher, indicating the sensor is detecting change.

When stable select 'OK'.

A message should be displayed confirming the calibration was 'successful'. Select 'okay' to return to the main scrolling page (this may happen automatically after a few seconds).

The change in the Tryptophan value can be seen in the data when the 1ml of stock solution is added whilst the graph continues to scroll during the process.

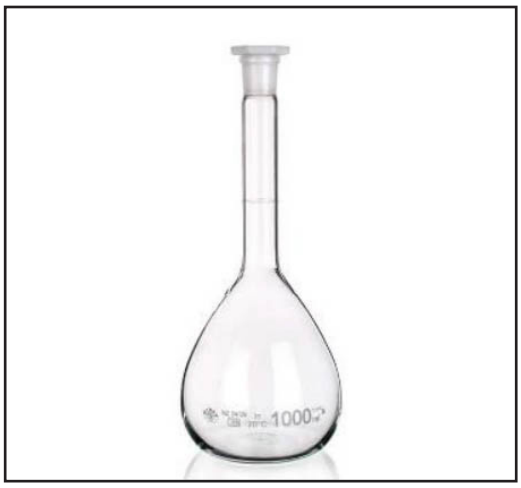
11. With the Proteus still submerged in the 100ppb fluid, review the values in the scrolling data. The Temperature Corrected value (TempCorr) should read 100ppb. The value obtained in stage 9 for the second calibration point should appear in the column 'TRYP ppb'. This parameter should be removed from the parameter list so it does not appear in the main scrolling page.

DATE	TIME	Temp deg C	pH units	pH mV	ORP mV	Stage m	HDO %Sat	Turb NTU	TRYP ppb	TRYP TempCorr
06/17/20	10:09:07	22.30	4.65	133.4	347.8	-0.006	105.8	0.12	89.34	98.16
06/17/20	10:09:06	22.30	4.65	133.4	348.3	-0.005	105.8	0.09	90.62	99.57
06/17/20	10:09:05	22.30	4.63	134.1	348.6	-0.005	105.8	0.12	91.90	100.96
06/17/20	10:09:04	22.30	4.63	134.4	349.2	-0.006	105.8	0.01	91.64	100.68
06/17/20	10:09:03	22.30	4.61	135.4	350.0	-0.006	105.8	0.11	90.88	99.84
06/17/20	10:09:02	22.30	4.67	132.1	351.1	-0.005	105.9	0.01	90.45	99.38

DATE	TIME	Temp deg C	pH units	pH mV	ORP mV	Stage m	HDO %Sat	Turb NTU	TRYP TempCorr
06/17/20	10:11:54	22.30	4.71	130.0	351.3	-0.006	105.7	0.09	99.31
06/17/20	10:11:53	22.30	4.70	130.1	351.3	-0.006	105.7	0.08	100.71
06/17/20	10:11:52	22.30	4.70	130.1	351.3	-0.006	105.7	0.07	99.86
06/17/20	10:11:51	22.30	4.70	130.0	351.3	-0.005	105.7	-0.02	100.39

Appendix

Equipment Examples



1 Litre volumetric

1 ml pipette

250 ml Squeezy Bottle

Weigh Dish/Boat

Laboratory Scales

Magnetic Stirrer