



CALIBRATION GUIDE Fluorescein

OBJECTIVE

Procedure for calibrating the FLUORESCEIN sensor.

PREPARATORY STEPS

- **Check**

Ensure that the FLUORESCEIN sensor has been correctly assigned to the AUX socket number it's fitted to. Not applicable if using AP/AS-PRO. Ensure that the probes end cap is clean and fitted during calibration and when taking measurements in the field. Failure to use the end cap will lead to incorrect calibration and erroneous readings in the field.

- **Required Items**

- FSCEIN-CAL (0.5g of fluorescein powder, add 1L of deionised water to make solution)
- Accurate pipette, 0-20ml range.
- Volumetric flask, 1L.
- Pure water (for example mineral water). This must have a conductivity of over 5uS/cm.

- **Preparation**

The probe and pure water should be left on the bench overnight so that their temperatures can equilibrate.

- **Equipment**

- Aquaprobe.
- Calibration instrument; Aquameter / Bluelink / Aquacal PC software
- FSCEIN-CAL solution.
- Volumetric flask, 1L
- Accurate pipette, 0-20ml range.
- Weigh boat

- **Serial Dilution**

Step 1; Weigh out 0.5g Fluorescein dye powder in a weigh boat and add to 1L pure water in a volumetric flask. Invert 10 times or until all powder is dissolved. This gives a stock solution of 500mg/L.

Step 2; Transfer 10ml of the 500mg/L stock solution into a 1L volumetric flask and top the flask up to 1L with pure water. Invert to mix. This step results in a 1 in 100 dilution of the 500mg/L stock resulting in a 5mg/L stock.

Step 3; Transfer 20ml of the 5mg/L stock from step 2 into a 1L volumetric flask. Top up to 1L with pure water. Invert to mix. This step results in a 1 in 50 dilution and gives you the 100 μ g/L FSCEIN calibration standard required for Point 2 calibration.

The value output by the probe directly after calibration should be approximately 100 μ g/L at 20°C (this value will vary with temperature). The dilute solution can be stored in a dark bottle in a refrigerator for up to five days. After that time it must be discarded.

CALIBRATION

Calibration guidance will assume you are using an Aquameter, procedure will be similar when using Bluelink or AquaCal.

Procedure, 0 calibration:

1. Pour the pure water down the side of the calibration vessel so that air bubbles aren't introduced to the liquid. Ensure there is at least 10cm of liquid in the calibration vessel.
2. Put the probe into the pure water. If the probe does not have a cleaning arm, tap the probe on the bottom multiple times to dislodge bubbles, after you have put it in the liquid and right before starting the calibration. If your probe does have a wiper, run a clean cycle after you put it in the liquid and right before starting the calibration.
3. Leave the probe to sit in the water for two minutes to stabilise.
4. To start the calibration, press the MENU key then select Calibration>Full Cal>AUX ELETRODES>FSCEIN to enter the FSCEIN calibration screen.
5. Move the cursor downwards to point 1, which is the 0 calibration.
6. Press the OK button on the Aquameter to begin the calibration process.
7. A calibration report value will be displayed after the calibration is complete, it is useful to record these values for troubleshooting purposes.

Procedure, 100 μ g/L calibration:

1. Pour the 100 μ g/L Fluorescein dilution down the side of the calibration vessel so that air bubbles aren't introduced to the liquid. Ensure there is at least 10cm of liquid in the calibration vessel.
2. Put the probe into the Fluorescein dilution. If the probe does not have a cleaning arm tap the probe on the bottom multiple times to dislodge bubbles, after you have put it in the liquid and right before starting the calibration. If your probe does have a wiper, run a clean cycle after you put it in the liquid and right before starting the calibration.
3. Leave the probe to sit in the Fluorescein dilution for two minutes to stabilise.
4. To start the calibration, press the MENU key then select Calibration>Full Cal>AUX ELETRODES>FSCEIN to enter the FSCEIN calibration screen.
5. Move the cursor downwards to point 2, which is the 100 μ g/L calibration.
6. Press the OK button on the Aquameter to begin the calibration process.
7. A calibration report value will be displayed after the calibration is complete, it is useful to record these values for troubleshooting purposes.

CONTROL

Check the calibration report value is within the acceptable range.

The acceptable range for the 0% calibration is >2200mV

The acceptable range for the 100 μ g/L calibration is >50mV above zero point

Once calibration is complete press the escape button until you can see the live readings. Check that the FLUORESCEIN readings is displaying a value **close to 100 μ g/L** at 20 degrees C. This value will vary with temperature.



OPERATING MODE

Dated	Revision	Document evolutions	Author
03/07/25	0	Creation	CP
04/07/25	1	Review and fine adjustment	GP

Notes