



**GGUN FL-24**  
**GGUN FL-30**

**Fluorometer**

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Calibration

## Optics definition

- I uranine, pyranine, eosine, chlorophyll A (optional)
- II amidorhodamine G, sulforhodamine B, rhodamine WT, resorufin
- III Tinopal CBS-X, CBS-CL, amino-G-acid , photine CU
- IV duasyn brilliant yellow T
- V naphthionate

## Calibration

Calibration is advisable 2-3 times a year, permitting proper system operation.

FL24 calibration is done by immersing the probe. For the FL30, simply pour the liquid into the probe, after removing the 2 caps and installing a stopper.

Calibration is done by using known standard solutions:

### 1. clean water

2. **tracers at  $10^{-7}$  g/ml** (Caution: use the same product for calibration and tracer test. There are significant differences between manufacturers!). Possibly calibrate at the temperature of the tracer test.

FL30 probe: To reach the optical cell, it is necessary to remove the two cylindrical caps. Both are secured with three screws. Close the lower inlet of the glass tube with the provided rubber stopper. Gently pour in the calibration solution to prevent bubble formation (very important!). Put one of the caps on the top to prevent daylight from entering during the measurement.

All probes:

Rinse one or two times (even more for Tinopal). Copy the measured mV data into your calibration file (CALIBRAT.DAT) without modifying its format. Example:

```
-----Full calibration of tracer 1-----
2      Number of calibration lines, increasing concentration
-8  28.70    log(ppb) & signal (mV)
-7  278.05   log(ppb) & signal (mV)
-----Full calibration of tracer 2-----
2      Number of calibration lines, increasing concentration
-7   16.80   log(ppb) & signal (mV)
-6  160.81   log(ppb) & signal (mV)
-----Full calibration of tracer 3-----
2      Number of calibration lines, increasing concentration
-7  100.44   log(ppb) & signal (mV)
-6 1014.87   log(ppb) & signal (mV)
```

In this Table, tracers 1, 2 and 3 could be uranine, rhodamine and Tinopal assigned to optics 1, 2 and 3. There is a line indicating the number of concentrations used for calibration (here, 2). The line starting with [-8 28.70...] says that for a concentration of tracer 1 of  $10^{-8}$ , lamp 1 gives a signal of 28.70 mV. Only lines with  $10^{-7}$  are needed. It is not advisable to use more than 3 concentrations (numerical instability).

Above  $10^{-6}$  g/ml, signal non-linearity may occur. Consequently, if accurate readings are necessary at higher concentrations, calibration should be extended and calibration lines added at  $10^{-6}$  and  $10^{-5}$  g/ml (not true for uranine saturating above 200 ppb).

Also edit the 100 ppb value in the 1st part of the table (with Notepad or Wordpad. Do not use Word):

-----Tracer #1-----	
Uranine	
L1	278.05
L2	3.75
L3	7.45
L4	540.20
-----Tracer #2-----	
SrhadaminB	
L1	18.40
L2	16.80
L3	1.42
L4	536.50
-----Tracer #3-----	
Tinopal-CL	
L1	1.16
L2	0.46
L3	100.44
L4	543.51
-----Turbidity-----	
1 NTU	
L1	1.00
L2	0.42
...	

The FLUO programme will use the polynomial interpolation only when one tracer is selected. If more than one is selected, then the calibration will assume a linear response and use only the response for the 100 ppb concentration given in the above Table.

The calibration utility CAL30 can also be used outside of the FLUO programme. This utility allows for fluorometer calibration. Prepare 100 ppb standards for tracers and 1, 10, 100 NTU for turbidity. For tracers use also a set of concentrations between 1 ppb and 10 ppm (2 contiguous concentrations such as 10 and 100 ppb are sufficient). Simple calibration is used when several (2 or 3) tracers are present in the water. The mathematical separation is based on linear equations. In this case, the fluorometer response to tracer concentration is supposed linear, and thus, only one concentration (100 ppb) is used in the calibration. A second calibration is necessary for more accurate polynomial interpolation of the fluorometer response. However, this method works only for solutions of one tracer. Water calibration is also important. Use degassed distilled or microfiltrated water (to avoid air bubbles).

To increase the precision of small turbidity measurements, it is sufficient to recalibrate only the water response.

## Nephelometric method - NTU reference

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Formazine suspension as turbidity standard

### **Solution 1**

1 g hydrazine sulphate  $(\text{NH}_2)_2\text{H}_2\text{SO}_4$  in 100 ml water

### **Solution 2**

10 g hexamethylene tetramine  $(\text{CH}_2)_6\text{N}_4$  in 100 ml water

### **End solution**

100 ml solution 1 plus 100 ml solution 2. Leave for 24 hours at 25 °C.

The turbidity of this solution is by definition 4000 NTU.

The solution is stable for 1 year in the dark.

### **Tap water**

Turbidity threshold target: 0.02 NTU. Achieved in tap water filtered with 0.1 micron pore size filter.