



## Quick-Start Guide

### Pall mPath™ IoR Concentration Monitor

This guide presents basic work to install, set up, and run the mPath IoR concentration monitor. If any step is not clear, or any of the criteria are not met, consult the operating and maintenance manual (Pall literature number USD 3341).

#### Before Installing: Key Environmental Requirements

- Ambient temperature 20 to 30 °C
- Process fluid temperature 5 to 50 °C
- Line pressure 0 to 5.5 barg
- Index of refraction (IoR) of process fluid 1.28 to 1.40  $n_D^{20}$
- The digital display unit (DDU) must be placed within 3 m of sensors, due to cable length

4. Connect the signal cable (black) to the back of the DDU with the RS232 (9-pin) connector. Use a slotted screwdriver to secure, hand tight.
5. Connect the signal cable (black) to the computer with the USB connector.
6. Connect the power cable to the DDU to turn on the monitor.

**Note:** *The DDU is always on when connected to the power supply.*

#### Install the Monitor

1. Open and clean the flow cells with an appropriate solution (such as 70% isopropanol) if they have been previously used, ensuring that the sensor is dry before reassembly. Cleaning may be verified by close inspection of the optical window surface using a light. Ensure there are no visible spots or smears on the optical window.
2. Connect the sensor cables (white) to the flow cells with the IP67 (12-pin) connector, finger-tight.
3. Connect the sensor cables (white) to the front of the DDU with the HD15 (15-pin) connector. Use a slotted screwdriver to secure, hand tight.

#### Set Up the Monitor

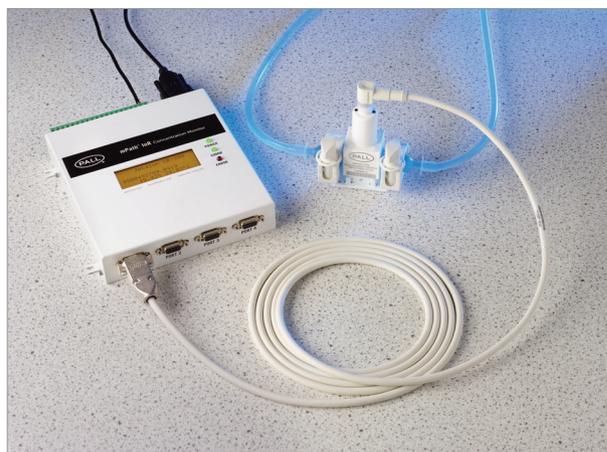
7. Launch 288-Connect software.
8. Under the <Display> tab, select <Auto Open> to set the communication port. If communication cannot be established, restart the DDU to establish communication.
9. Access sensor settings by clicking the <About> box and enter the password <Advanced288>.
10. Under the <Sensor Calibration> tab, press <Load Memory> to import sensor settings.
11. Under the <Factory Calibration> tab, press <Initialize Sensor>.
12. Under the <Diagnostics> tab, select the <TIR Curve> view.
13. Ensure that TIR Curve is flat; the “Line Quality in Air” should be below 3.0<sup>-3</sup>.

14. Select **<Save Memory>** to export sensor settings.
15. Repeat steps 10 to 14 with each sensor being used.
16. Connect flow cells to process fluid lines (refer to Figure 1).

**Note:** Flow cells should be placed upstream of pumps, and downstream of pressure-regulating devices.

**Note:** Flow cells can be mounted in various orientations depending on the desired level of bubble detection and the risk of sedimentation on the optical window.

17. Flush lines with a fluid with a known refractive index (such as deionized water [ $n_D = 1.333000$ ]).
18. Under the **<Sensor Calibration>** tab, press **<Load Memory>** to import sensor settings.
19. Under the **<Factory Calibration>** tab, set the **<Rezero Reference IoR>** value and press **<Rezero Sensor>**.
20. Select **<Save Memory>** to export sensor settings.
21. Repeat steps 18 to 20 with each flow cell being used.
22. As observed under the **<Data Graph>** tab, at steady-state the temperature should vary by not more than  $\pm 0.1$  °C and the IoR signal should vary by not more than  $\pm 2^{-6} n_D^{20}$  ( $1.332998$  to  $1.333002 n_D^{20}$  for water).



## Run the Monitor and Log Data

23. With the sensor initialized in air and calibrated on a known liquid, begin data collection by selecting the **<Data Logging>** tab.
24. Select desired data collection rate from the drop-down box: 1 sec, 5 sec, 10 sec, 30 sec, 1 min, or 5 min.
25. Tick **<Also Save Existing Data>** box if desired.
26. Select **<Start Now>** when ready to begin data logging.

**Figure 1**

Three orientations for the flow cells for different levels of bubble detection



Normal orientation for minimal sedimentation



Vertical orientation, recommended



Inverted orientation for minimum bubble detection

## Generating a Standard Curve

To enable the use of IoR as a reference quantity for protein concentration, a standard curve must be generated for each protein and in each buffer matrix. The relationship between IoR and protein concentration will be linear ( $y = mx + b$ ) within a wide range which covers all typical bioprocessing concentration ranges. The slope ( $m$ ) is dictated by the protein refractive index coefficient, while the y-intercept ( $b$ ) is dictated by the background refractive index of the buffer matrix.

1. Prepare samples of various known protein concentrations in the buffer matrix. For statistical significance, it is recommended that a 5-point standard curve is generated. The limits of this curve should frame the anticipated concentrations of the application.
2. Ensure that the sensor is initialized in air, calibrated on a known liquid, and that data is being logged.
3. Begin flowing one of the prepared samples through the sensor.

**Note:** It is common to begin with lower concentration samples and move to higher concentrations.

4. Hold the sensor at steady-state for 10 minutes to collect several data points at the known concentration.

5. Flush the sensor with water and hold steady-state for 2 minutes.
6. Repeat steps 3 to 5 for all prepared concentration samples.
7. Retrieve the logged data. This is saved as a comma-separated values (.csv) data file. Use a spreadsheet program (such as MS Excel<sup>♦</sup>) to generate a plot of the known concentrations on the x-ordinate against the IoR response on the y-ordinate.
8. Generate a linear regression to find the gradient. This can then be applied to unknown concentrations of the protein in the same buffer matrix.

**Note:** When presenting data, it is sometimes desirable to eliminate unnecessary decimal places. This may be done with IoR values by using water normalization. For example: two IoR values of 1.337455 n and 1.339747 n can be simplified by subtracting the IoR of water (1.333000) to be 0.004455 ṅ and 0.006747 ṅ, respectively. These water-normalized IoR values may then be expressed with metric prefixes to be 4455 μṅ and 6747 μṅ, which has been found to yield data in a more user-friendly format.



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