Azura

Air sensor
Supplement

HPLC

ℹ **Note:** Please read the corresponding technical documentation for handling and safety reasons.

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Intended use

The air sensor displays the end of buffer or the end of sample by detecting air. It protects the column from damage caused by intruding air and supports the automatic sample injection. The air sensor can be used with different transparent 1/16", 1/8" or 1/4" tubings.

The delivery consists of the air sensor, an amplifier, and a gameport adapter. The gameport adapter can be connected to a computer.

The air sensor is controlled by KNAUER software PurityChrom® which supports up to 4 air sensors. Upon detecting air, you can program different actions. The purification can either be stopped or paused to prevent air from entering the system. Furthermore, after detecting the end of the sample, you program the software to automatically start or continue the run.

The LEDs of the amplifier indicate the status of the air sensor:

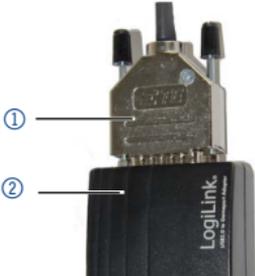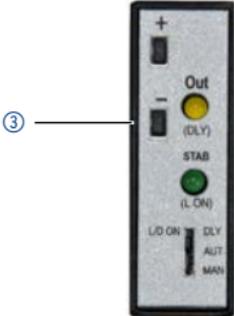| LED color	Figure
Yellow LED Out ①: ■ Lights up in case air has been detected **Green LED STAB ②:** ■ Lights up permanently to show that the signal is stable ■ In case of flickering, calibration is recommended	

Installation

See below for a description on how to mount the air sensor onto the tubing and on how to connect it to a computer. You can attach the air sensor to the side panel of your AZURA® L device with AZURA® Click. Select a position for the air detection on the tubing, then start the installation.

ℹ️ **Note:** The number of the air sensor corresponds to the number on the cable that is being used. If you have more than one air sensor, connect the other amplifiers to the free cables.
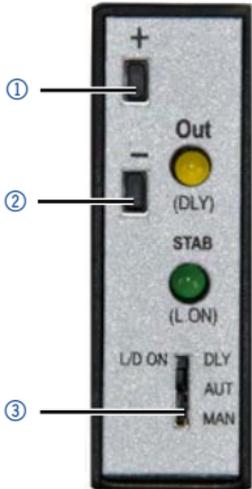
Process	Figures
1. Connect the Sub-D15 plug ① with the gameport adapter ② . **2.** Connect the gameport adapter per USB to your computer.	① ②
3. Connect the amplifier ③ to one of the 4 numbered cables.	③
4. Clamp the air sensor ④ onto the tubing at the selected position.	④

Calibration

You must calibrate the air sensor before use or in case the signal is unstable. For that purpose, make sure that you can operate the amplifier.

> **ⓘ** **Note:** During calibration, the green LED STAB blinks rapidly. If calibrating has been successful, the same LED blinks slowly for approximately 3 s.

Process	Figure
1. Move the switch ③ to DLY. 2. Press the - button ②. The STAB signal disappears. Both lights should be turned off. 3. Move the switch to AUT. 4. Press the + button ① where the tubing is filled with air. 5. Press the button - where the tubing is filled with liquid.	

Integrating into PurityChrom®

You can program the software to either start or stop the system after an air bubble has been detected.

> **ⓘ** **Note:** You have to choose the *Gameport Input 1, 2, 3* or *4* which equals the air sensor number.

Process and figures

1. Open the *PurityChrom® Setup*.
2. Go to the register *Communication*.
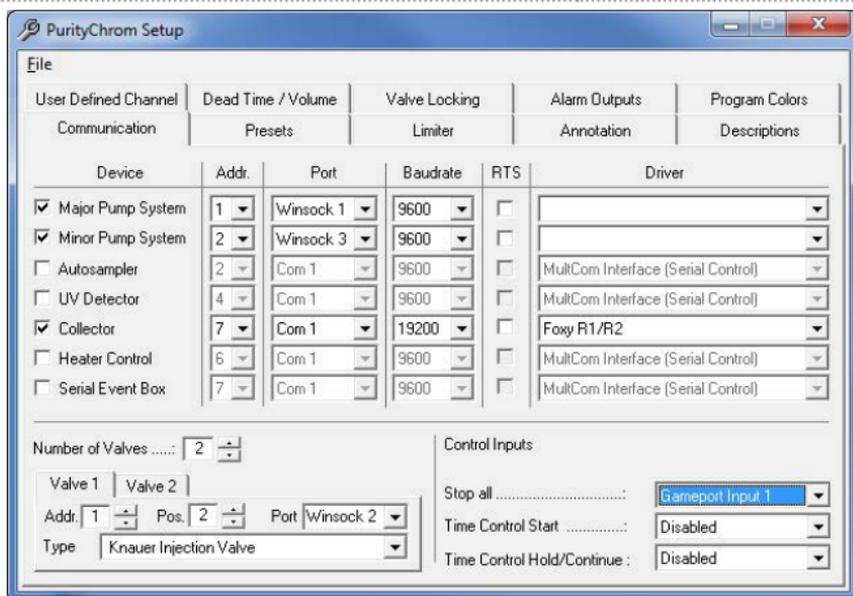3. Make the necessary changes in the section *Control Inputs*.

Fig. 1: PurityChrom® Setup

Process and figures	
4. To stop the system, change the settings as follows:	Control Inputs Stop all ....: Gameport Input 1 Time Control Start ....: Disabled Time Control Hold/Continue : Disabled
5. To start a run, change the settings as follows:	Control Inputs Stop all ....: Disabled Time Control Start ....: Gameport Input 1 Time Control Hold/Continue : Disabled

Holding the run

To hold the run at air detection, you can program a threshold over your complete run in the register *Threshold* of the *Time Control Editor*.
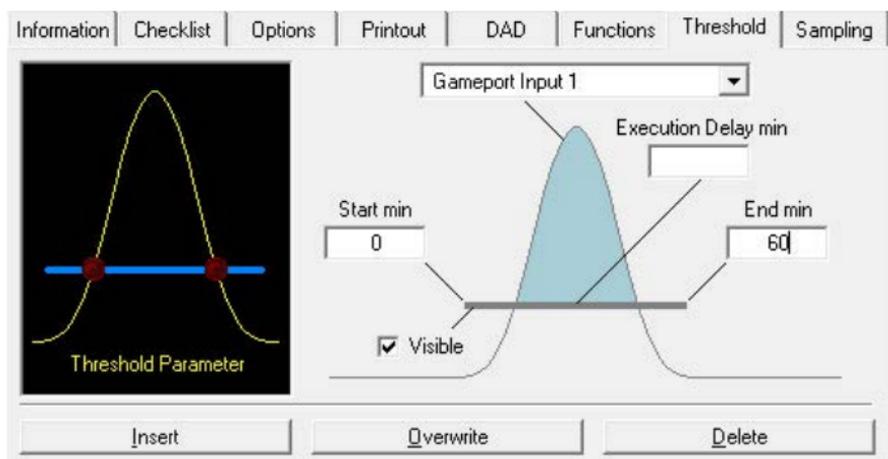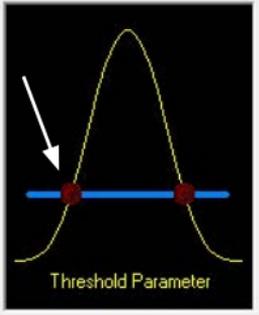

Fig. 2: Register *Threshold*

> **Note:** You have to choose the *Gameport Input 1, 2, 3* or *4* which equals the air sensor number.

Process	Figures
1. To open the *Time Control Editor*, press the button 🖼.	
2. Go to the register *Threshold*.	
3. Choose the *Gameport Input* 1 from the dropdown list.	
4. Click on the start value of the threshold parameter in the picture (indicated by the arrow). The appearance of the register changes.	Threshold Parameter
5. Change the setting for *Operation* to *Hold current run*.	Device ...... PurityChrom
6. Click on the button *Insert* to confirm the changes.	Operation ... Hold current run
	Parameter ..
	Insert Overwrit

Continuing the run

To continue the run after air has been detected, you can activate the setting in the register *Functions* of the *Time Control Editor*.
Start writing your method as usual and activate the function *Wait for Input Signal* at a particular time during the method.

Fig. 3:   Register *Functions*

ⓘ **Note:** Make sure that the functions succeeding the function *Wait for Input Signal* start with a delay of 0.01 s.

ⓘ **Note:** Do not activate the option *Stop Pumps* at *Time Control Hold* in the register *Options*. Otherwise the pump stops after the function *Wait for Input Signal* has been reached.

ⓘ **Note:** You have to choose the *Gameport Input 1, 2, 3* or *4* which equals the air sensor number.

Process	Figures
1. To open the *Time Control Editor*, press the button .	
2. Go to the register *Functions*. **3.** Activate the function *Wait for Input Signal*. **4.** Choose the *Gameport Input 1* from the dropdown list.	Input Signal Gameport Input 1
5. Activate *On* in the section *State*. **6.** Click on the button *Insert* to confirm the changes. **7.** After reaching the function *Wait for Input Signal*, the run pauses until the end of sample. After the signal, the run continues automatically.	State ◯ Off ◉ On ◯ Pulse
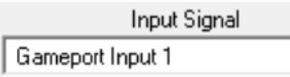
Air sensor usage for sample application with the sample pump (with chromatogram)

The best way to implement the sample application with an airsenor for varying sample amount is the usage of the variable "*Time Shift*".
A variable is an unknown value in a method which will be set just before the run, which makes your method flexible and adjustable.

In this case it means that upon starting the method you will be asked how much sample you want to load onto the column just before running the method. The length of sample application in your method is adjusted accordingly.

Process and figures

1. Write your time control file in volume or sample volume with the presumption of sample application being a small amount of sample e.g. half the volume of the column.

2. Important for the start of sample application via the pump is:

   a. The injection valve is put in "direct load".

   b. Flow rate and composition of the sample pump is set as intended.

   c. The major pump flow rate is set to 0 ml/min (Fig. 4).

   d. A threshold for the respective airs ensor is inserted with start point being start of sample application and end point being stop of sample application (Fig. 5).

   e. The "threshold over event" is put to jump to next line (Fig. 6).

Column	Function	Parameter	
0.00	Composition Major Pump	100.0 , 0.0	
0.00	Composition Minor Pump	100.0	b
0.00	Flowrate Major Pump	0.00 ml/min Constant Flow	c
0.00	Flowrate Minor Pump	2.00 ml/min Constant Flow	b
0.00	Valve Position	Multi-Injection Valve = Direct Load	a
0.00	Threshold	0.50 / Gameport Input 3	d
0.02	Start Chromatogram	Channel 1,2,3,4,P,P (500 ms)	
0.02	UV Autozero		
0.50	Flowrate Major Pump	2.00 ml/min Constant Flow	
0.50	Flowrate Minor Pump	0.00 ml/min Constant Flow	
0.50	Valve Position	Multi-Injection Valve = Manual Load	
2.00	Composition Major Pump	100.0 , 0.0	

Fig. 4: Time Control File indicating the parameters for sample application using the sample pump.
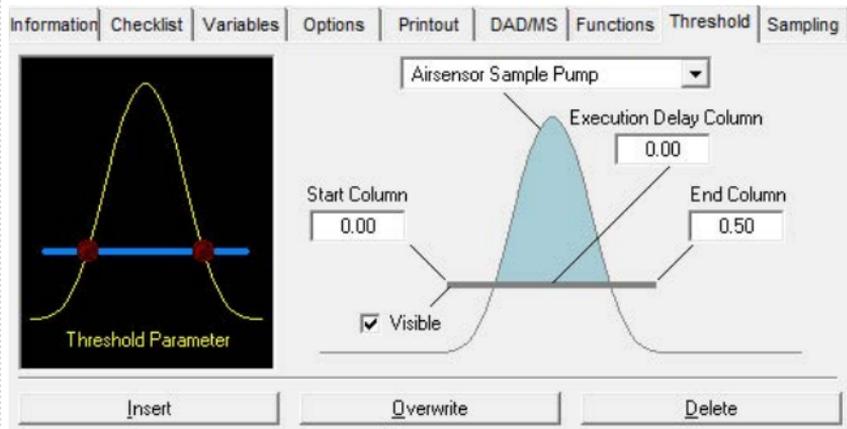
Process and figures

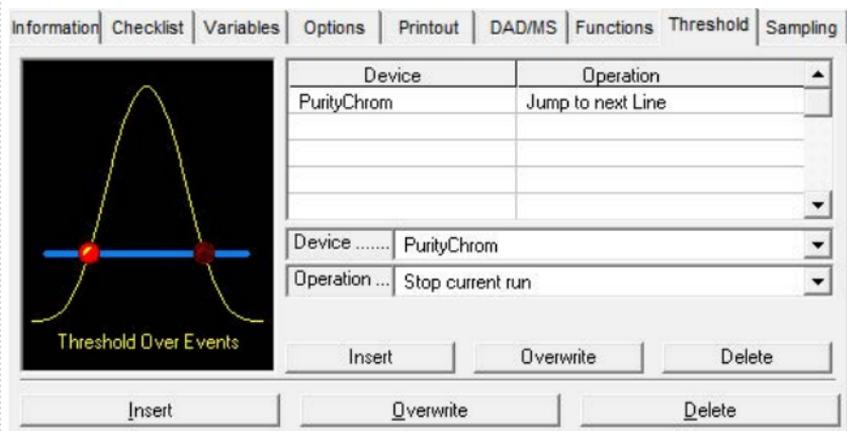**Fig. 5:** Threshold parameters for sample application surveillance via the air sensor

Fig. 6: Threshold function which is taken upon air detection

Process and figures

3. At the time point of stopping the sample application (in this example 0.5 CV) following functions must be set

- **a.** The injection valve needs to be set back to "*manual load*"
- **b.** The wanted flow rate of the major pump needs to be set
- **c.** The flow rate of the minor pump needs to be set to 0 ml/min.

Column	Function	Parameter	
0.00	Composition Major Pump	100.0 , 0.0	
0.00	Composition Minor Pump	100.0	
0.00	Flowrate Major Pump	0.00 ml/min Constant Flow	
0.00	Flowrate Minor Pump	2.00 ml/min Constant Flow	
0.00	Valve Position	Multi-Injection Valve = Direct Load	
0.00	Threshold	0.50 / Gameport Input 3	
0.02	Start Chromatogram	Channel 1,2,3,4,P,P (500 ms)	
0.02	UV Autozero		
0.50	Flowrate Major Pump	2.00 ml/min Constant Flow	b
0.50	Flowrate Minor Pump	0.00 ml/min Constant Flow	c
0.50	Valve Position	Multi-Injection Valve = Manual Load	a
2.00	Composition Major Pump	100.0 , 0.0	

Fig. 7: Time Control File indicating the parameters after sample application

4. To be able to adjust the volume of the sample being applied the "*Time Shift*" variable needs to be used.

5. Go to the *Variable tab* and insert the variable with the default value at 0.02 units earlier than the stop of sample application (Fig. 8). The default value defines a time point, after which all defined functions will be shifted by the time/volume. (Fig. 9)

ⓘ | **Note:** The time shift default value needs to be at least 0.02 earlier than the first function to be carried out after air is detected because all functions with the exact same time of the default time shift value are not shifted.

Process and figures

Fig. 8: Variables tab with *„Time Shift"* variable setting

Column	Function	Parameter
0.00	Composition Major Pump	100.0 , 0.0
0.00	Composition Minor Pump	100.0
0.00	Flowrate Major Pump	0.00 ml/min Constant Flow
0.00	Flowrate Minor Pump	2.00 ml/min Constant Flow
0.00	Valve Position	Multi-Injection Valve = Direct Load
0.00	Threshold	0.50 / Gameport Input 3
0.02	Start Chromatogram	Channel 1,2,3,4,P,P (500 ms)
0.02	UV Autozero	
0.50 + Sample	Flowrate Major Pump	2.00 ml/min Constant Flow
0.50 + Sample	Flowrate Minor Pump	0.00 ml/min Constant Flow
0.50 + Sample	Valve Position	Multi-Injection Valve = Manual Loa
2.00 + Sample	Composition Major Pump	100.0 , 0.0
10.00 + Sample	Composition Major Pump	50.0 , 50.0
10.02 + Sample	Composition Major Pump	0.0 , 100.0
15.00 + Sample	Composition Major Pump	0.0 , 100.0
15.00 + Sample	Stop all	

Fig. 9: Time Control File after setting the variable *„Time Shift"* in the variable tab

Process and figures

6. Upon starting the method, the volume of the sample is specified (Fig. 10). This will lead to a shift of the gradient and all functions defined afterwards. (Fig. 11/Fig. 12).
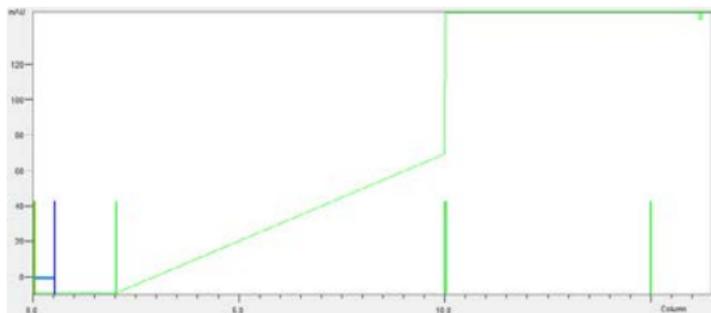

Fig. 10: Variables list at the start of a run

Fig. 11: Method with 0 CV sample size respective Time Shift = 0 CV. The blue horizontal bar indicates the threshold over the air sensor and therefore the time at which sample is applied
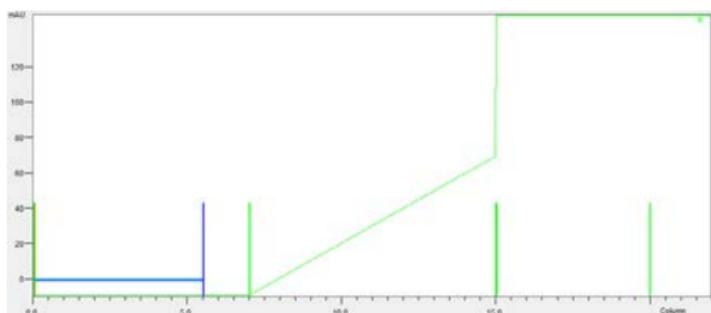
Process and figures

Fig. 12: Method with 5 CV sample size respective Time Shift = 5 CV. The blue horizontal bar indicates the threshold over the air sensor and therefore the time at which sample is applied. The method gradient is shifted back about the time

7. If the air sensor recognized air/end of the sample it will trigger the threshold, with the set function "*Jump to next line*" the method will go to the next line of the time control file. In this case the flow of the major pump starts, the flow of the minor pump stops, the valve is switched and the gradient and fractionation you set afterwards is executed.

ⓘ | **Note:** Always put in a small amount more volume than you have sample, to avoid too low amount of sample being applied to the column if imprecise volume measuring took place.

ⓘ | **Note:** Depending if you are writing the method in volume, column volume or time the default value unit is either in ml, CV or min.

Repeat orders

Name	Order number
Air sensor for 1/16″ tubing	**A70092**
Additional air sensor without wiring for 1/16″ tubing	**A70092-1**
Air sensor for 1/8″ tubing	**A70093**
Additional air sensor without wiring for 1/8″ tubing	**A70093-1**
Air sensor for 1/4″ tubing	**A70083**
Additional air sensor without wiring for 1/4″ tubing	**A70083-1**
AZURA Click	**A70096**
Distribution box 24 V	**AZS80SA**

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