

## Two-parameter Monitoring in Lab-on-valve Manifold, Applied to Intracellular H<sub>2</sub>O<sub>2</sub> Measurements

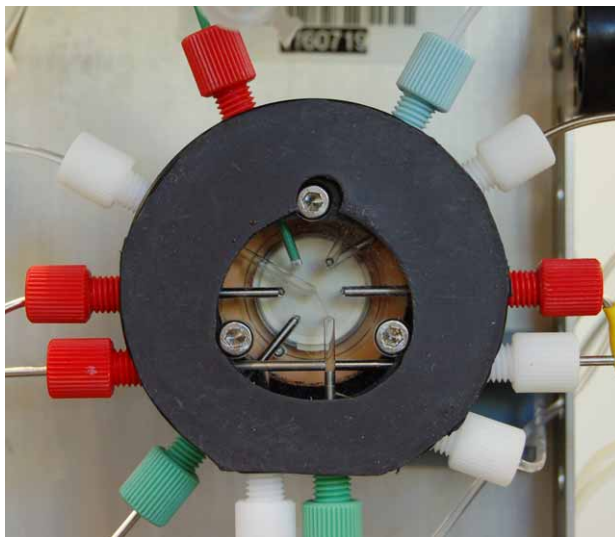
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### LOV photograph



A photograph of the Lab-on-valve unit with black coating for fluorescence measurements. The clear part of the front face allows visual inspection of the flow cell and the LOV channels. The black part of the front face has been painted, and the paint overlaid with a piece of magnetic strip. This allows covering the clear part by placing a magnetic disk against the front face.

## Software protocol

### ' SETTINGS.

#### ' Valve port settings.

Hardware Settings portname (number, string) 1, waste  
Hardware Settings portname (number, string) 2, sample  
Hardware Settings portname (number, string) 3, beads  
Hardware Settings portname (number, string) 4, flowcell  
Hardware Settings portname (number, string) 5, DCF

#### ' Spectrometer settings for absorbance measurements.

Hardware Settings Wavelength 1 (nm) 530  
Hardware Settings Wavelength 2 (nm) 470  
Hardware Settings Integration Time (msec) 5  
Hardware Settings Detectors to Average 5  
Hardware Settings Samples to Average 20  
Hardware Settings Scan Rate (Hz) 1

#### ' Turn on LED.

Analog:Digital Module TTL Line Off (#) 3

#### ' LOAD BEAD COLUMN.

Analyte New Sample

#### ' Reference signal in an empty flowcell.

Spectrometer Reference Scan

#### ' Fill syringe.

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 250  
SyringePump Delay Until Done

#### ' Aspirate in beads.

SyringePump Valve Out  
Valve beads  
SyringePump Flowrate (microliter/sec) 20  
SyringePump Aspirate (microliter) 100  
SyringePump Delay Until Done

#### ' Start recording absorbance signal.

Spectrometer Absorbance Scanning

#### ' Dispense beads into flowcell.

SyringePump Flowrate (microliter/sec) 10  
Valve flowcell

SyringePump Empty  
SyringePump Delay Until Done

#### ' Flush bead inlet. Discard possible excess beads to waste.

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 500  
SyringePump Delay Until Done  
SyringePump Valve Out  
Valve waste  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Dispense (microliter) 200  
SyringePump Delay Until Done  
Valve beads  
SyringePump Flowrate (microliter/sec) 20  
SyringePump Dispense (microliter) 50  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 50  
Valve waste  
SyringePump Empty  
SyringePump Delay Until Done

#### ' Wash bead column.

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 200  
SyringePump Delay Until Done  
SyringePump Valve Out  
Valve flowcell  
SyringePump Flowrate (microliter/sec) 10  
SyringePump Empty  
SyringePump Delay Until Done

#### ' Stop recording absorbance signal. Turn off LED.

Spectrometer Stop Scanning  
Analog:Digital Module TTL Line On (#) 3

#### ' STAIN CELLS.

#### ' Prime DCFH-DA port.

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 300  
SyringePump Delay Until Done  
SyringePump Valve Out  
Valve DCF  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 60  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 50  
Valve waste

SyringePump Empty  
SyringePump Delay Until Done

' **Inject DCFH-DA.**

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Aspirate (microliter) 500  
SyringePump Delay Until Done  
SyringePump Valve Out  
Valve DCF  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 500  
SyringePump Delay Until Done

' **Deliver DCFH-DA onto cells, switch to intermittent flow for staining.**

Valve flowcell  
SyringePump Flowrate (microliter/sec) 10  
SyringePump Dispense (microliter) 50  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 2  
Loop Start (#) 30  
    SyringePump Dispense (microliter) 5  
    SyringePump Delay Until Done  
    Delay (sec) 3  
Loop End  
Valve waste  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Empty  
SyringePump Delay Until Done

' **EXPOSE TO H2O2.**

' **Re-define spectrometer settings for fluorescence measurements.**

Hardware Settings Wavelength 1 (nm) 530  
Hardware Settings Wavelength 2 (nm) 470  
Hardware Settings Integration Time (msec) 100  
Hardware Settings Detectors to Average 5  
Hardware Settings Samples to Average 5  
Hardware Settings Scan Rate (Hz) 1

' **Prime H2O2 inlet.**

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Aspirate (microliter) 600  
SyringePump Delay Until Done  
SyringePump Valve Out  
Valve sample  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 100  
SyringePump Delay Until Done  
SyringePump Flowrate (microliter/sec) 100  
Valve waste  
SyringePump Empty

SyringePump Delay Until Done

' **Fill pump.**

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 100  
SyringePump Aspirate (microliter) 500  
SyringePump Delay Until Done

' **Aspirate in H2O2.**

SyringePump Valve Out  
Valve sample  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 500  
SyringePump Delay Until Done

' **Turn on LED. Start continuous recording of fluorescence signal. Deliver H2O2 onto cells. After 5 sec, stop continuous recording and turn off LED.**

Analog:Digital Module TTL Line Off (#) 3  
Spectrometer Voltage Scanning  
SyringePump Flowrate (microliter/sec) 5  
Valve flowcell  
SyringePump Empty  
Delay (sec) 5  
Spectrometer Stop Scanning  
Analog:Digital Module TTL Line On (#) 3

' **Trigger LED on/off, for pulsed recording of fluorescence signal. Note: the pump keeps delivering H2O2 all through these steps.**

Loop Start (#) 8

Delay (sec) 21  
Analog:Digital Module TTL Line Off (#) 3  
Spectrometer Voltage Scanning  
Delay (sec) 2  
Spectrometer Stop Scanning  
Analog:Digital Module TTL Line On (#) 3

Loop End

SyringePump Delay Until Done

' **Stop recording fluorescence signal. Turn off LED.**

Spectrometer Stop Scanning  
Analog:Digital Module TTL Line On (#) 3

' **DISCARD BEADS.**

' **Pull most of the beads out.**

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 400

SyringePump Delay Until Done

SyringePump Valve Out  
Valve flowcell

SyringePump Flowrate (microliter/sec) 10  
SyringePump Aspirate (microliter) 50  
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 10  
SyringePump Dispense (microliter) 10  
SyringePump Delay Until Done

SyringePump Flowrate (microliter/sec) 10  
SyringePump Aspirate (microliter) 150  
SyringePump Delay Until Done

Valve waste  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Empty  
SyringePump Delay Until Done

' **Push the remaining beads out.**

Loop Start (#) 2

SyringePump Valve In  
SyringePump Flowrate (microliter/sec) 50  
SyringePump Aspirate (microliter) 600  
SyringePump Delay Until Done

SyringePump Valve Out  
Valve flowcell

SyringePump Flowrate (microliter/sec) 300

Loop Start (#) 5

SyringePump Aspirate (microliter) 2  
SyringePump Delay Until Done  
SyringePump Dispense (microliter) 100  
SyringePump Delay Until Done

Loop End

SyringePump Flowrate (microliter/sec) 200  
SyringePump Empty  
SyringePump Delay Until Done

Loop End

Loop End