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SURFE²R N1 Protocols

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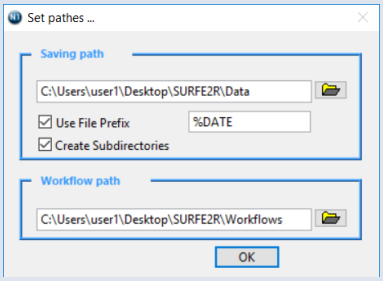
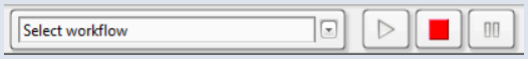
Quickstart Guide

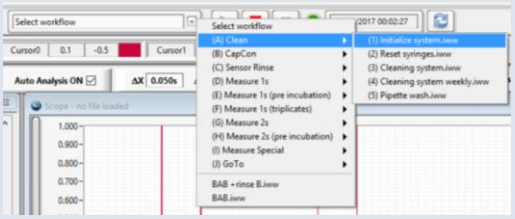
Start the system

Turn on the SURFE²R N1 device and start the software. Wait until initialization has finished and the software is ready. Set the saving path for your workflows and data. Confirm that it is correct and click "ok". Check that system water is available and the waste container is empty.



Select the workflow "(A) Clean (1) initialize system" in the drop down menu and start it using the arrow button to fill all tubes and syringe pumps with system liquid. This takes about 2 min.





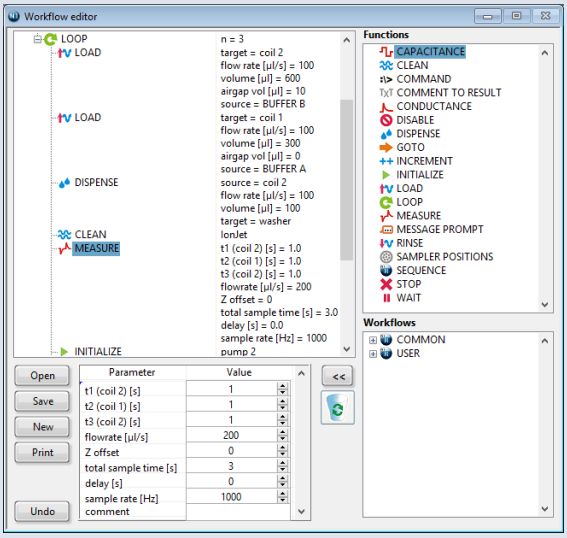
Hint

Checking the box "Create Subdirectories" will create a folder labelled with the date for this experiment. If you don't check it all files will be written in the same folder.

Choose a workflow

The workflow contains all the required information for the instrument to perform one experiment, either one single measurement or a whole sequence of measurements with up to 53 different solutions. The drop down menu contains pre-defined workflows at the top and custom-designed workflows at the bottom. The pre-defined workflows are described in the workflow documentation available in the help menu.

Customize a workflow. The workflow editor enables the researcher to define a specific experiment. Functions can be added to the workflow by drag & drop from the function list. The parameters can be set at the bottom when a function is selected in the workflow window. The settings include the duration, speed and volume of solution flow during the experiment, the number and sequence of different buffers, the number of repetitions per measurement, the incubation times between experiments and the volume used for rinsing the sensor after an experiment.



If you want to design your own workflows there are several points to consider:

- **It's not possible to take up more than 2 ml of each buffer.**
- **The aspirated volume must be 100 µl greater than the dispensed volume.**
- **If you use a loop, make sure that you don't run out of buffer. If air is pipetted on the sensor it will be ruined!**

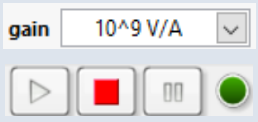
Set up an experiment

Position the buffers. Check in the workflow which positions of the probe sampler are in use and which buffer they are supposed to contain. Fill the vessels accordingly and make sure the spring of the Ionjet does not touch the liquid. Usually position one contains activating buffer, position two contains non-activating buffer and position three contains the resting buffer. This can be repeated up to position 53 when measuring under different conditions.

Mount a sensor and close the cage. The sensor only fits in one position: the socket of the sensor has to be positioned on the contact pin. Move the locking to the left over the sensor and mount the black shielding cap (Faraday cage).

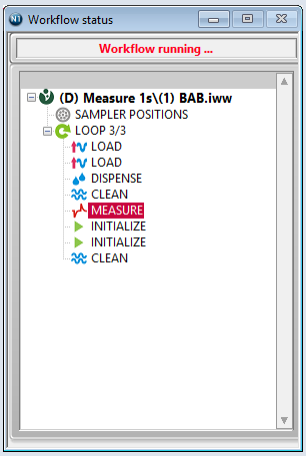
Set the cursors in the software. For automated data analysis set the cursors accordingly (see data handling). Peak currents then will be detected and added to the results sheet documentation.

Set the gain of the amplifier. Usually the gain is set to 10^9 V/A to resolve currents up to 10 nA. If signals are > 10 nA or < 1 nA, the amplification can be adjusted to 10^8 V/A and 10^{10} V/A respectively.



Run the experiment

Start and pause an experiment: The measurement itself generally consists of loading and injecting the solutions as well as washing the pipette and tubing after each sequence. Everything is fully automated and takes about 3 min for each measurement. Start the measurement by clicking on the play button. During the measurement the green indicator will flash red. The progress of the workflow can be viewed in the workflow status window. You can pause and stop the run by clicking on the corresponding buttons. The probe sampler will not stop right away but the ongoing step will be finished first.



Sequence of measurements: Depending on the number of single measurements and incubation times in between, the SURFE²R N1 can work independently for several hours. Be sure to include incubation times between measurements with different buffer compositions to adjust the intraliposomal volume to the new buffer. When measuring one sensor using different buffer conditions and the aim is to observe the change in the peak current, always use a rundown control: Check for sensor stability over the time of the measurement by recording peak currents at the beginning and the end of the sequence using the same buffers.

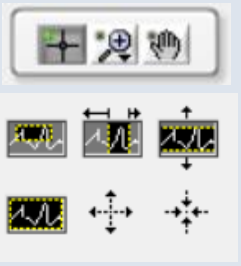
Finish for the day

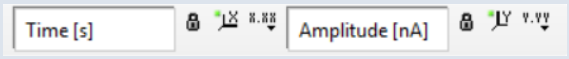
After finishing all experiments for the day, select the workflow "(A) Clean (3) Cleaning system" for daily maintenance. Follow the instructions on the screen: The system will be sequentially flushed with 30% isopropanol or 30% ethanol and water. Afterwards the tubing is emptied. Close the software and shut down windows. The system will turn off automatically.

You should run the workflow "Cleaning system" daily and the workflow "Cleaning system weekly" before weekends. This is very important since it prevents the formation of a biofilm which will cause noise and artifacts.

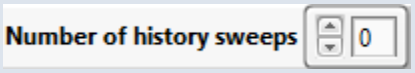
The graph window

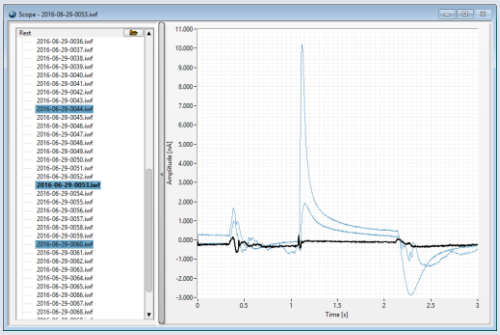
Navigating: If the options menu "Scope: Drag and Zoom" is activated, you can zoom in via right click drag and drop on the current trace. If deactivated an additional bar appears showing a **cross**, a **magnifier** and a **hand**. The hand allows moving the position of the coordinate system. The magnifier allows zooming in and out using different options. The cross enables the manual movement of the cursors. To get back to **full scale** click the x and y buttons beside the axis titles on the upper panel. They scale the x and y axis to full range. The **lock** symbol allows fixing the current view of the graph window; When activated the scale of the graph window stays the same, also when new traces are recorded.

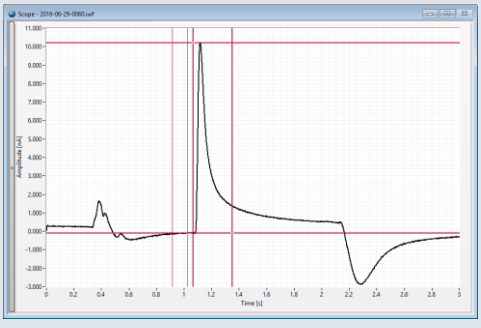




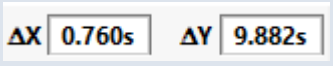
Comparing different traces: During the measurement a number of current traces recorded before the present measurement can be overlaid by setting the "Number of history sweeps". The latest trace is shown in black, previous traces are shown in light blue. Any recorded traces can be compared directly by overlaying in the graph window. Click on the small arrow at the left side of the graph window to extend the data browser. Click the file names you want to overlay while holding Ctr.





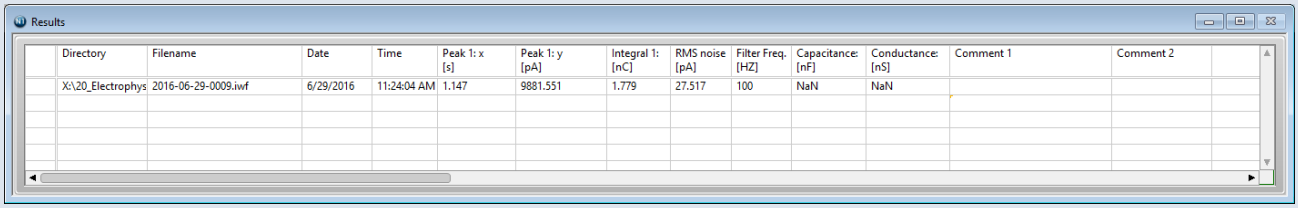


Cursors: The positioning of the cursors can be used to manually measure distances. These are shown as Δx and Δy in the upper panel of the software.

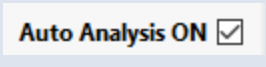


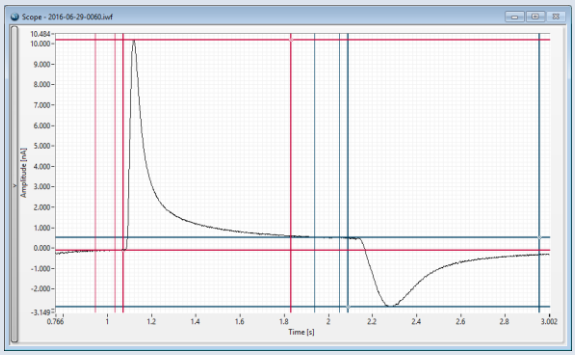
Data handling

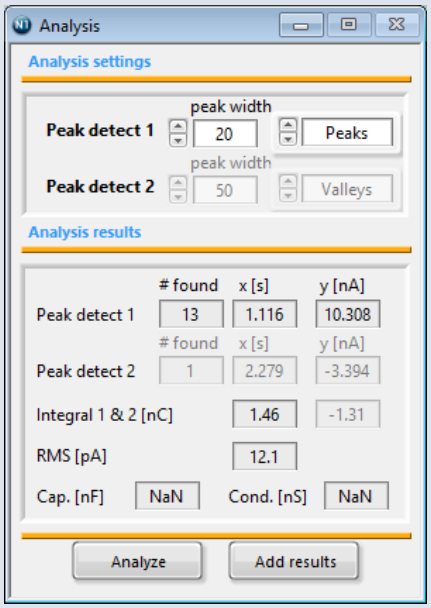
Results sheet: The results sheet is saved as text file. It contains meta data for each trace. This information includes filename, time of measurement, peak currents, integral, capacitance, conductance and columns for comments like experimental conditions or workflow. Information for peaks and integrals depend on the setting of the respective cursors. The contents of the results sheet can be customized by clicking "customize" in the right click menu of the sheet.



Automated analysis: Peaks and integrals can be calculated automatically during the measurement when "auto analysis on" is activated. Set the cursors appropriate to define the intervals for the detection of the baseline (thin lines) and the peak current or integral (thick lines). Red and blue lines represent two different set of cursors, e.g. for analyzing on and off signals separately. Blue cursors can be disabled in the options menu. Inside the analysis window the peak width (number of averaged data points defining the peak current) and the polarity of the peak have to be set.







Batch Analysis: To analyze peak currents or integrals after the measurement, set the cursors in the graph window and the peak width and peak polarity in the analysis window. Then click "batch analysis" in the file menu and select the files you want to analyze. The results are shown in the results sheet.

Data export: To export selected traces chose export ASCII from the file menu.

Sensor Preparation & Cleaning

Materials

N1 Single Sensors:	Nanion, Nr. 2-03-35002-000
Lipid Solution:	1,2-diphytanoyl-sn-glycero-3-phosphocholin, n-decane
Thiol Solution:	1-octadecanethiol, isopropanol or ethanol
Membrane preparation:	Containing the protein of interest
Non-activating Buffer:	Specific for each transporter – check assay protocol
Ultrasonic processor:	Tip sonicator (UP 50 H, Dr. Hielscher, equipped with MS 1 tip) or Bath sonicator (Sonorex RK 52 H, Bandelin)
Centrifuge:	Suitable for plates or 50 ml Falcons

We strongly recommend the use of lipid from AVANTI POLAR LIPIDS. All chemicals should be ultra-pure, since contamination might disturb the measurement.

Preparation of Thiol Solution

Prepare a 0.5 mM solution of 1-octadecanethiol in isopropanol.

For 100 ml: Take 14.33 mg 1-octadecanethiol.

Use a glass bottle and slightly heat and stir the solution until the thiol is dissolved completely. Avoid evaporation of the solvent. Store the solution in the dark at room temperature and check for precipitates before use. If necessary, reheat carefully to dissolve precipitates.

Preparation of Lipid Solution

Prepare 7.5 µg/µl 1,2-diphytanoyl-sn-glycero-3-phosphocholin in n-decane.

For 3.33 ml: Take 25 mg of the lipid.

When the lipid is dissolved in chloroform, remove the solvent using a rotary evaporator. The lipid dissolves immediately in n-decane. Transfer the solution in a glass bottle with a Teflon lid, and store at -80°C or -20°C. Always allow the bottle to warm up to room temperature before opening to avoid water condensation forming on the inside of the bottle.

The thiol and lipid solutions must not be stored or prepared in plastic vials! Use glass vials only, ideally with a teflon lid or inlay.

Preparation of Membranes

- Rapidly thaw an aliquot of the membrane preparation, e.g. proteoliposomes or membrane vesicles from cell culture or native tissue.
- Prepare a homogenous membrane suspension by diluting the membranes with non-activating buffer to yield the optimized lipid or total protein concentration for the adsorption to the sensor. The dilution factor has to be optimized empirically for each membrane preparation; usually dilutions between 1:5 and 1:100 are suitable.
- Sonicate the membrane suspension in a 1.5 ml Eppendorf tube. For proteoliposomes use gentle sonication with a bath sonicator. Use 3 to 5 sonication cycles, each lasting 10 s and put the membranes on ice in-between to prevent damage by overheating. For membrane vesicles use a tip sonicator by applying 10 bursts with an amplitude of 20% and a cycle time ratio of 0.5.
- After sonication immediately add the membranes to the sensor.

Sensor Preparation

Sensor preparation can be done in batches, e.g. 10 sensors at the same time.

Thiolation of the sensor surface

- Fill the sensor well with 50 µl of thiol solution.
- Incubate for at least 30 min at room temperature in a closed petri dish. This incubation can be done overnight.
- Remove the solution by tapping the sensor upside-down on a tissue.
- Rinse the sensor three times with isopropanol or pure ethanol.
- Rinse the sensor three times with deionized water.
- Dry the sensor thoroughly by tapping it on a tissue. Be sure the sensor surface is free of water droplets.

Assembly of the lipid layer

- Apply 1.5 µl of the lipid to the surface of the thiolated sensor without touching the gold surface with the pipette tip.
- Immediately fill the sensor well carefully with 50 µl of non-activating buffer to spontaneously form the SSM.

Application of Membranes on a SURFE²R Sensor

- Apply 5 to 10 µl of the diluted and sonicated membrane preparation by submerging the pipette tip in the solution covering the sensor. Slowly dispense the suspension onto the sensor surface. Do not touch the surface of the sensor with the pipette tip.
- Centrifuge the sensor for 30 min at 2000 to 3000 g. Use a plate centrifuge with suitable adapter or put the sensors in 50 ml falcon tubes.
- You can use the sensor immediately or incubate the sensor at 4°C for several hours or overnight. Make sure that the sensor does not dry out, e.g. by sealing the well with a tape or by placing the sensors in an air-tight box or a box with a damp tissue.

The SURFE²R sensors can be stored after preparation at 4°C for 1 to 3 days without significant loss of activity. If kept at –80°C, they may be ready-to-use for weeks or even months. This is mainly limited by the stability of the protein.

Check for sensor quality

- The sensor quality can be checked at different stages during sensor preparation, e.g. after forming the SSM and before adding the membrane preparation. This way, bad sensors can be discarded before adding the membrane preparation. It's worth adding an incubation time of ≥ 30 min (or overnight) between SSM formation and quality control. This yields more stable values for capacitance and conductance of the membrane. In addition, quality control should take place right before starting the measurements. At the end or during the measurement the sensor quality can be checked to prove the sensor stability.
- Position the socket of the sensor on the contact pin located on top of the device. Move the locking to the left and shield the sensor using the Faraday cage.
- Measure capacitance and conductance by choosing the respective workflow in the software. The workflow "CapCon" measures both sequentially.
- Compare the output (results sheet) with ideal values (see table). When the SSM is not formed correctly capacitance and conductance are higher resembling values of the pure gold sensor. To check the instrument itself the parameters of a test chip can be used as a benchmark.
- When the parameters do not fulfill the requirements, the sensor should be discarded.

	Capacitance	**Conductance**
ideal sensor with SSM	15 – 30 nF	< 5 nS
pure gold sensor	~ 75 nF	~ 40 nS
test chip	10 nF	2 nS

Sensor Cleaning

The SURFE²R sensors were originally designed for single use only. Nanion does not guarantee sensor quality when being reused. Be aware that mechanical contact produces scratches. Due to glued parts, sonication is not possible.

- Rinse the sensor with 10 ml water using a pipette and dry it by tapping the sensor on a tissue or using pressurized gas.
- Fill the sensor with 100 µl isopropanol or ethanol and incubate for about 10 min.
- Pre-soak a cotton swab (q-tip) with isopropanol or ethanol and gently rotate the cotton swab on the sensor surface without applying pressure to the sensor.
- Rinse the sensor sequentially with 5 ml pure isopropanol or ethanol and 10 ml water.
- The sensor should dry over-night to prevent corrosion. Check the surface for scratches and cleanliness the next day. Store the sensor under inert gas for a longer life-time.

Sample Requirements

Your samples are precious, and so is your time. SURFE²R measurements can yield extremely interesting data, but there are pitfalls, some of which you can avoid from the very start.

Source material: Sample preparation commonly begins either with recombinant overexpression in eukaryotic cell lines (CHO, HEK and COS-1), bacteria or yeast or with the isolation of membranes from native tissue such as heart, kidney, liver, brain, skeletal muscle or gastric mucosa from different organisms including pig, rabbit or mice. Cell free expressed transporters in nanodiscs as well as whole cells have been used for adsorption to the SSM, but usually purified samples are required.

It is highly unlikely that crude homogenates or lysates will give any protein-specific signals. Sample preparation is the key to successful measurements, since a high protein density and purity can compensate for low turnover, low electrogenicity and large solution exchange artifacts.

Membrane purification: When using isolated membrane samples, after cell disruption the membranes should be purified using a sucrose density gradient centrifugation. This is especially appropriate when membranes from distinct organelles like mitochondria, ER, synaptic vesicles or lysosomes are required. It is also commonly employed for the enrichment of plasma membrane fragments. Sucrose gradient centrifugation yields a significant signal enhancement compared to non-purified samples.

Protein reconstitution: High turnover transporters usually work with membrane preparations. But they show lower signal to noise compared to reconstituted samples. The ideal sample therefore is prepared by protein purification followed by reconstitution into liposomes at high protein densities. Transporters with turnover rates below 100 s^{-1} should be characterized using proteoliposomes with low lipid-to-protein ratio (LPR) of 5 to 10. The LPR of successfully tested proteoliposomes range from 5 (LacY, turnover of 30 s^{-1}) to 500 (NhaA, turnover of 1000 s^{-1}).

When using reconstituted samples it is critical to ensure that no residual detergent remains in the membrane preparation after reconstitution. Different procedures are described for detergent removal, e.g. rapid dilution or incubation with Bio-Beads®. If detergent remains in the protein sample, the sensor can be destroyed during the adsorption process.

Sample Concentration: The lipid concentration has to be optimized before starting the experiments. Start with a dilution sequence of the sample and check for the signal amplitudes. For your experiments then use a sample concentration achieving high signal amplitudes with the lowest sample consumption possible. Too high concentrations as well as too low concentrations can reduce the signal amplitude.

The sample can be diluted in the respective non-activating buffer directly before the measurement. Usually lipid concentrations between 0.2 mg/ml and 5 mg/ml are used. In the case of membrane preparations with unknown lipid concentration, the total protein concentration can be used as a benchmark. Typical samples are prepared with 2 mg/ml to 10 mg/ml total protein concentration. For adsorption to the SSM total protein concentrations between 0.1 and 1 mg/ml are used. Since the concentration of the protein of interest cannot be adjusted directly, the output mainly depends on the expression efficiency which, therefore, also has to be optimized.

Sample Volume: For each sensor 5 to 10 µl of the diluted sample is required. If the sample amount is critical this volume could be reduced even further. Therefore 50 to 100 µl could be enough for a rough characterization of the transporter. Depending on the measurement sequence it takes up to a few days to measure 100 µl of the sample.

Assay Development

Negative control membrane: During assay development it is important to also test a negative control membrane. This can be empty liposomes or a membrane preparation without the protein of interest. The solution exchange can induce a transient current also in the negative control membrane due to interactions of the charged buffer components with the membrane. The current obtained with the negative control membrane roughly reflects the artifact fraction of the current obtained for the membrane containing the protein of interest. The artifact fraction should be less than 10 % of the transporter current.

Buffer preparation: If large artifacts are observed, the buffers should be optimized to reduce the artifacts. This can be done by reducing the concentration of the substrate or using high salt background concentrations. Also make sure that activating and non-activating buffers are prepared in the same batch to make them as similar as possible. Divide the solutions afterwards and add the substrate to the activating solution. Often in the non-activating solution a compound is used compensating the substrate in the activating solution. In a sodium transporter assay 10 mM NaCl can be used in the activating solution, while 10 mM KCl is used in the non-activating solution. Also the used concentrations can differ to yield the lowest artifacts.

Activity tests: Ideally, protein activity of an aliquot of the sample has already been determined prior to the SURFE2R experiment, e.g. by means of a photometric assay or an uptake assay (positive control). This is especially suitable when the electrogenicity is not known and no currents are observed. By knowing the transporter is functional in the respective sample, problems with the sample can be excluded. Also radioactive or fluorometric transport assays could help in interpreting the transient currents recorded with the SURFE2R N1 device, especially if it's not clear if the signal is due to binding or transport.

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Workflow Tree

Name of Workflow
Assignment of solution positions to positions in the autosampler, e.g. buffer A is placed in position 1 of the autosampler.
Number of different experiments
3 min incubation in buffer C before measurement
Number of experiments for each buffer system
Loading of non-activating buffer (B) into coil 2 of the IonJet
After valve switching, loading of activating buffer (A) into coil 1 of the IonJet
Dispensing of 100 µl non-activating solution into so washing station (to start solution flow at the sensor with B) and cleaning of the IonJet
Measurement consists of sequential solution flow of the solutions defined as B (from coil 2), A (from coil 1) and B (from coil 2), each 1.0 s. Simultaneous trace recording. The flow of A activates the transporter.
Resetting pump 2. Then loading of buffer C from the reservoir and injecting to the sensor to wash of the solution B.
Washing the coils with water, resetting the syringes, cleaning the IonJet.
Redefining the sampler positions: the next buffer A is placed in position 1+3 = 4 of the autosampler.

(E) Measure 1s (pre incubation)	
SAMPLER POSITIONS	BUFFER A = 1 BUFFER B = 2 BUFFER C = 3 REF A = 4 REF B = 5 REF C = 6
LOOP	n = 2
RINSE	flow rate [µl/s] = 100 rinse vol [µl] = 1000 source vol [µl] = 1100 source = BUFFER C Z offset = 0
WAIT	n [s] = 180
LOOP	n = 1
LOAD	target = coil 2 flow rate [µl/s] = 100 volume [µl] = 600 airgap vol [µl] = 10 source = BUFFER B
LOAD	target = coil 1 flow rate [µl/s] = 100 volume [µl] = 300 airgap vol [µl] = 0 source = BUFFER A
DISPENSE	source = coil 2 flow rate [µl/s] = 100 volume [µl] = 100 target = washer
CLEAN	IonJet
MEASURE	t1 (coil 2) [s] = 1.0 t2 (coil 1) [s] = 1.0 t3 (coil 2) [s] = 1.0 flowrate [µl/s] = 200 Z offset = 0 total sample time [s] = 3.0 delay [s] = 0.0 sample rate [Hz] = 1000
INITIALIZE	pump 2
RINSE	flow rate [µl/s] = 100 rinse vol [µl] = 1000 source vol [µl] = 1100 source = BUFFER C Z offset = 0
INITIALIZE	pump 2
INITIALIZE	coils
CLEAN	IonJet
INCREMENT	BUFFER A = 3 BUFFER B = 3 BUFFER C = 3 REF A = 0 REF B = 0 REF C = 0

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document version: 05-2017