

New cleaning protocols for MicroCal VP-DSC system and MicroCal VP-Capillary DSC system

New cleaning protocols are described for effective cleaning of DSC cells containing high concentrations of aggregated proteins. These cleaning protocols are considerably safer than a concentrated nitric acid solution that is currently used in many laboratories.

The new cleaning solution is made of 5% sodium hydroxide (NaOH) and a sodium hypochlorite (NaOCl) solution.

Preparation of cleaning solution:

- 1 Weigh five grams of sodium hydroxide (pellets, ACS reagent grade) and dissolve with 25 ml of de-ionized or distilled water in a 100 ml volumetric flask.
- 2 Add 50 ml of sodium hypochlorite solution (reagent grade, containing ~ 10-15 % chlorine) to the flask.
- 3 Add de-ionized or distilled water to the mark (i.e., 100 ml).
- 4 Mix the solution well.

Storage:

This solution will be effective for at least one year if stored in a dark, airtight container. If left on the instrument in the clear bottle provided it should be replaced every 3 months.

Cleaning procedure for MicroCal VP-DSC system

MicroCal VP-DSC system has a characteristic baseline when both cells are clean and filled with water. Repeatable baselines are indicative of clean, well filled cells.

Cleaning procedures

A. Normal cleaning

- 1 Make sure that the system has cooled to below 50°C.
- 2 Remove the denatured protein solution with the filling syringe.
- 3 Soak the cell with 20% Contrad 70 at 50°C for 10 minutes.
- 4 Cool the cell to room temperature and rinse thoroughly with distilled or de-ionized water.
- 5 After cleaning, perform a buffer vs. buffer or water vs. water run to make sure that the cell is properly cleaned. If not use the rigorous cleaning procedure.

B. Rigorous cleaning

In extreme cases when the protein has formed gelatin-like or glue-like protein aggregates that cannot be removed by the filling syringe.

- 1 Add the cleaning solution in small aliquots and heat to 50°C for a few minutes until the aggregate starts to dissolve.
- 2 Repeat until all the aggregated protein in the cell is completely dissolved, as judged from the color of the removed solution.
- 3 Cool the cell to room temperature and rinse well with distilled or de-ionized water.
- 4 After cleaning, perform a buffer vs. buffer or water vs. water run to make sure that the cell is properly cleaned.

Note: *Improper cell loading could also cause the baseline to be irregular and should be practiced before committing valuable material to a VP-DSC experiment.*

Cleaning procedure for MicroCal VP-Capillary DSC system

MicroCal VP-Capillary DSC system has a characteristic baseline when both cells are clean and filled with water. Repeatable baselines are indicative of clean, well filled cells.

The three bottles on the solvent reservoir station of the Auto Sampler should be filled with water (i.e., "SolvRes1"), 20% Contrad 70 (i.e., "SolvRes2") and the cleaning solution (i.e., "SolRes 3"), respectively. Both bottles for the rinsing solution should be filled with de-ionized water.



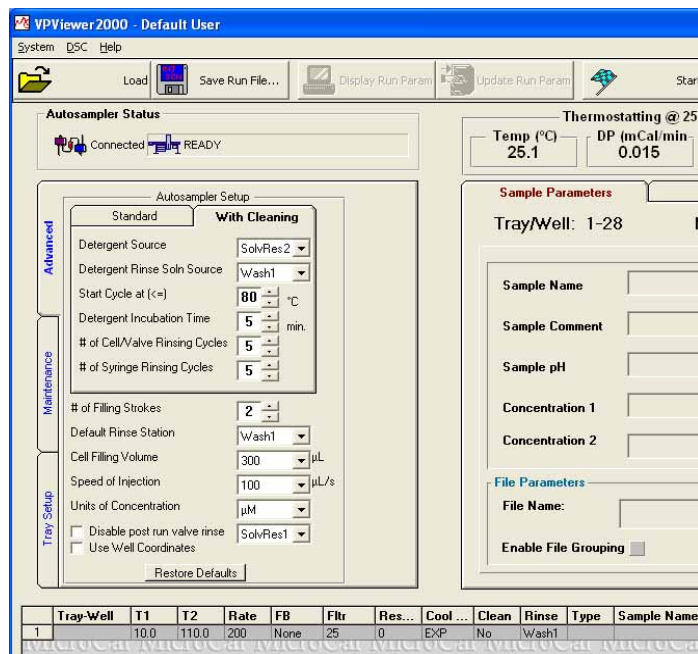
1 Set up cleaning by clicking the **Advanced** tab on the left hand side of the **Autosampler Setup** box and enter the following parameters:

- **Detergent Source:** SolvRes2
- **Detergent Rinse Soln Source:** Wash 1
- **Start Cycle at (<=):** 80°C
- **Detergent Incubation Time:** 5 mins
- **# of Cell/Valve Rinsing Cycles:** 5
- **# of Syringe Rinsing Cycles:** 5
- **# of Filling Strokes:** 2
- **Default Rinse Station:** Wash 1
- **Cell Filling Volume:** 300 µl
- **Speed of Injection:** 100 µl/s

Note: *Units of Concentration* does not impact on the cleaning.

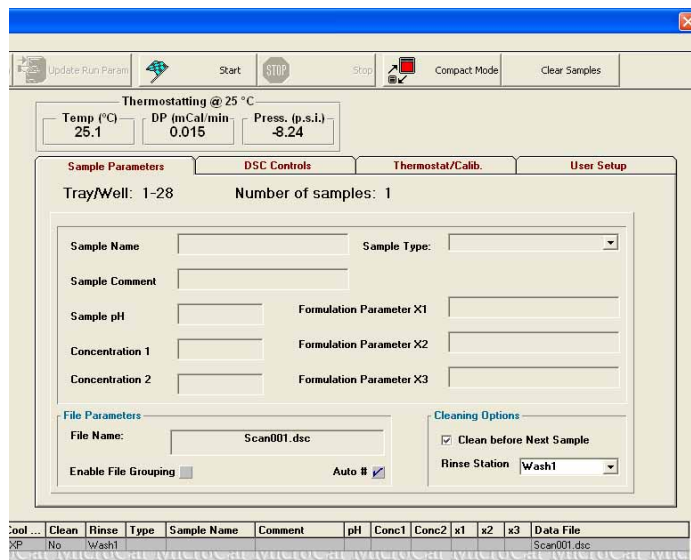
Note: Do not **Disable the post run valve rinse** with SolvRes 1.

If you are using a software version older than 2.0, these options can be found under the **Auto Sampler** tab followed by clicking **Advanced** and **With Cleaning** tab.



2 Click on **Sample Parameters** and check the **Clean Before Next Sample** box for each sample run.

If you are using version 1.4 of the software, the clean command can be activated by clicking on the **Tray Set up** tab and activating the **cln** box in the **Sample List**.



3 If this cleaning is not sufficient, use step 1 with SolvRes3.

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