

AUTOITC200 QUICK REFERENCE OPERATION PROTOCOL

This protocol serves as a guideline to help you design and conduct your experiments. Please familiarize yourself with the *MicroCal Auto-iTC200 User's Manual* (abbreviated as [The Manual](#) in the following texts) before starting your experiments.

EXPERIMENTAL DESIGN

See [Section 4.2: Experimental Design of The Manual](#) (page 49-50) for detail

SAMPLE PREPARATION

See [Section 2: Sample Preparation of The Manual](#) (page 20-26) for detail

>Follow the [Sample Preparation](#) guideline that was sent to you.

SET UP EXPERIMENTS ON THE COMPUTER

See [Section 3: Autosampler Software of The Manual](#) (page 27-45) for detail

>The [System](#) tab

Under the “Tray Temperature Controller” heading, check the current tray temperature. To change temperature, input desired temperature in the box named “Set Point”, and then click “Set”. If you are using the TubeRack, then do the same with the “TubeRack Temperature Controller”.

>The [Instrument Setup](#) tab

ITC Method - All available ITC methods are displayed in the table to the left. To add your own method, input your parameters in the boxes to the right, and save your method.

>The [Sample Groups](#) tab

>If you have done the EXPERIMENTAL DESIGN, you should know how many groups of samples you have. A Group of samples will have the same Automation Method, ITC Run method, and Analysis Method. So, if you know your samples require different methods, then they belong to different groups.

>In addition to your sample groups, we require two control sample groups, one before your first sample group, and one after your last sample group. These are for the EDTA control experiments.

EXAMPLE OF GROUP 1 SETUP

This is the EDTA control experiment before your samples. The main purpose is to make sure the system is operating normally, and if any error occurs, it will not cause the loss of your samples.

Dataset Naming

| | | | | | |
|-------------------|----------|-----------------|---|--------|--------------|
| Number of Samples | 1 | | | | |
| Prefix | EDTActrl | Starting Number | 1 | Suffix | Today's date |

Methods

| | |
|-------------------|-----------------|
| Automation Method | Plates Prerinse |
| ITC Run Method | EDTA25inj13 |
| Analysis Method | OneSite |

Concentrations

| | |
|----------------------------|-----|
| Cell Concentration (mM) | 0.2 |
| Syringe Concentration (mM) | 2.5 |

Plate Setup

| | | | |
|---------------|---|------|----|
| Starting Well | | | |
| Plate | 1 | Well | A1 |

----- END OF EXAMPLE -----

>Set up other sample groups following the example above. Note: we require two EDTA control experiments to be run in the second control sample group. Please use “Plates Prerinse Extra Clean” as the automation method for the first EDTA control after your samples.

>The Experiments tab

>Click Import Sample Groups to load the sample information into the spreadsheet.

>You may change the Data Path to your own folder.

>You may change the file name for the Out Log, so that this set of experiments will be added into the log file. The log file is an excel file that contains all the raw data and automatically analyzed results of your experiments.

>Click Validate to make sure there is no error. Pay attention to the amount of water, methanol and detergents the experiments require and the amount of waste that’ll be generated. Make sure there are plenty of solutions in the reagent bottles, and enough space in the waste bottle.

>Export the spreadsheet as an excel file so you can access it later.

LOAD THE SAMPLE TRAY

>Have the sample spreadsheet in the Experiments tab shown on the computer screen. You may scroll the spreadsheet to the right to show the columns titled “Cell Source”, “Pipette Source” and “Pre-rinse Source”. These columns tell you which wells your samples should go in the sample plates.

>You need 400 ul samples for the cell, and 140 ul samples for the syringe. You also need 400 ul matching buffer for the pre-rinse if you choose the method that requires pre-rinse.

>After making sure the samples are in their correct wells, seal the sample plate with the plastic cover.

>Put the sample plate in the sample tray. Make sure it’s loaded into the correct tray position (usually position “1” is selected). Close the tray.

NOW YOU ARE READY TO START THE EXPERIMENTS, BUT BEFORE YOU CLICK THE “START” BUTTON, PLEASE DO THE FOLLOWING:

>Ask core personnel to check your setup if you are not sure.

>Additional note: once the titration starts, the data will be displayed in the Data Plots tab.

WHEN YOUR EXPERIMENTS ARE FINISHED, THE RAW ITC FILES AND CORRESPONDING OPJ FILES WILL BE EMAILED TO YOU. YOU MAY COME AND PICK UP YOUR SAMPLE PLATES, OTHERWISE IT WILL BE STORED AT ROOM TEMPERATURE BY CORE PERSONNEL.

DATA ANALYSIS

See [Section 5: Origin of The Manual](#) (page 63-67) for detail

>Data analysis requires Origin software with the ITC custom package. If you don’t have this software, there is one computer available in Room 254 for you to perform data analysis, please schedule computer time with core personnel.

>Follow [Section 5.2 Origin Data Analysis Tutorial](#) to learn the main steps in data analysis.