SOP number	G001-02
Title:	Usage of SpectraMax photometer
Replaces SOP	G001-01
Changes	adaption to new SpectraMax ABS Plus Microplate reader and to SoftMax Pro 7.2 software
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Date	26.01.2024
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Date	01.02.2024
Signature	

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1. Description

This SOP describes the usage of the SpectraMax ABS Plus Microplate Reader.
The SpectraMax ABS Plus microplate spectrophotometer provides rapid and sensitive measurements of a variety of analytes across a wide range of concentrations. It measures the optical density (OD) of samples at selected wavelengths (190-1000 nm) in a number of reading modes:
Endpoint: at a single point in time
Kinetic: over a specified period of time
Spectral scan: over a specified wavelength range.
The SpectraMax ABS Plus reads 96-well plates, 384-well plates, and cuvettes. The contents of the wells in a microplate can be mixed before each reading cycle using the shaking function, which makes it possible to perform kinetic analysis of solid-phase and enzyme-mediated reactions (mixing is not critical for liquid-phase reactions).
The temperature of the microplate chamber can be regulated, if desired from 5°C above ambient to 45°C.
The SpectraMax ABS Plus in our institute is mainly used to determine the optical density (OD) of proteins and dyes coupled to antibodies (Abs) to calculate protein concentration and dye to protein coupling ratios as well as for ELISA and BCA experiments.

2. Abbreviations / Definitions

abbreviation	**description**
Abs	antibodies
nm	nanometer
OD	optical density
96 WP	96 well plate

3. Safety Precautions

Wear a lab coat during the preparation.

4. Material

Material	**supplier, order number**
Hellma® TrayCell® cuvette	Sigma, 105.800
Kimtech wipes	Kimtech, 05511

5. Procedure

- do not wear gloves when using the device
- first switch on the corresponding PC
- switch on the photometer by turning on the multiple socket outlet

NOTE: The PC must always be switched on first and then the photometer. Otherwise, the photometer will not be connected or recognized.

5.1 Measurement with the tray cell cuvette

- open SoftMax Pro 7.2 software 

- press the illuminated button on the front of the device to close the plate drawer
- press the button “New Cuvette Set” in the left

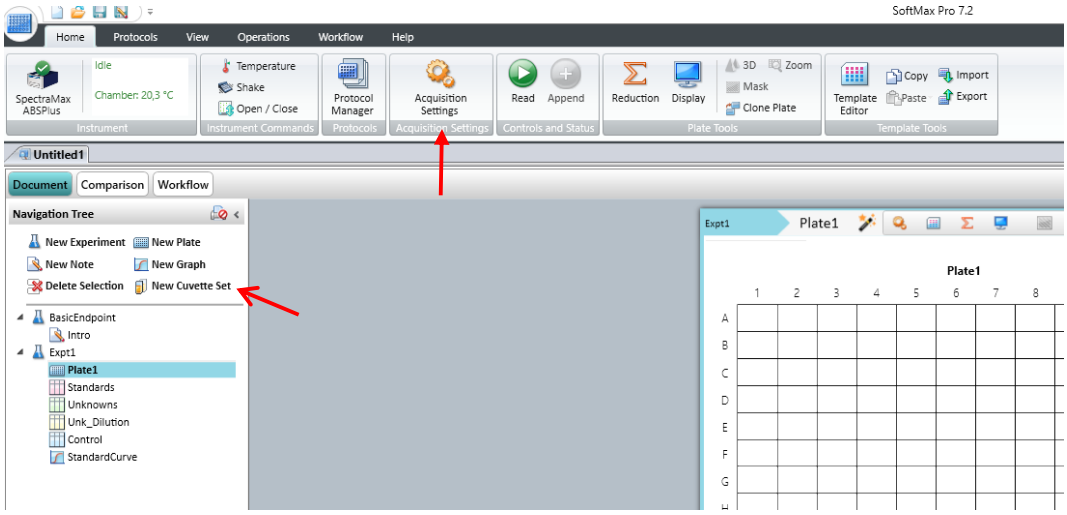

Figure 1: SoftMax Pro 7.2 start screen menu (see figure 1)

- press the button “Acquisition Settings” in the top menu to adjust the measurement settings

- select the number of wavelengths (max. 2 in parallel) that you require for your application (figure 2) by entering the numbers directly (Monochromator, adjustable in 1 nm steps)
- confirm the settings with the “ok” button

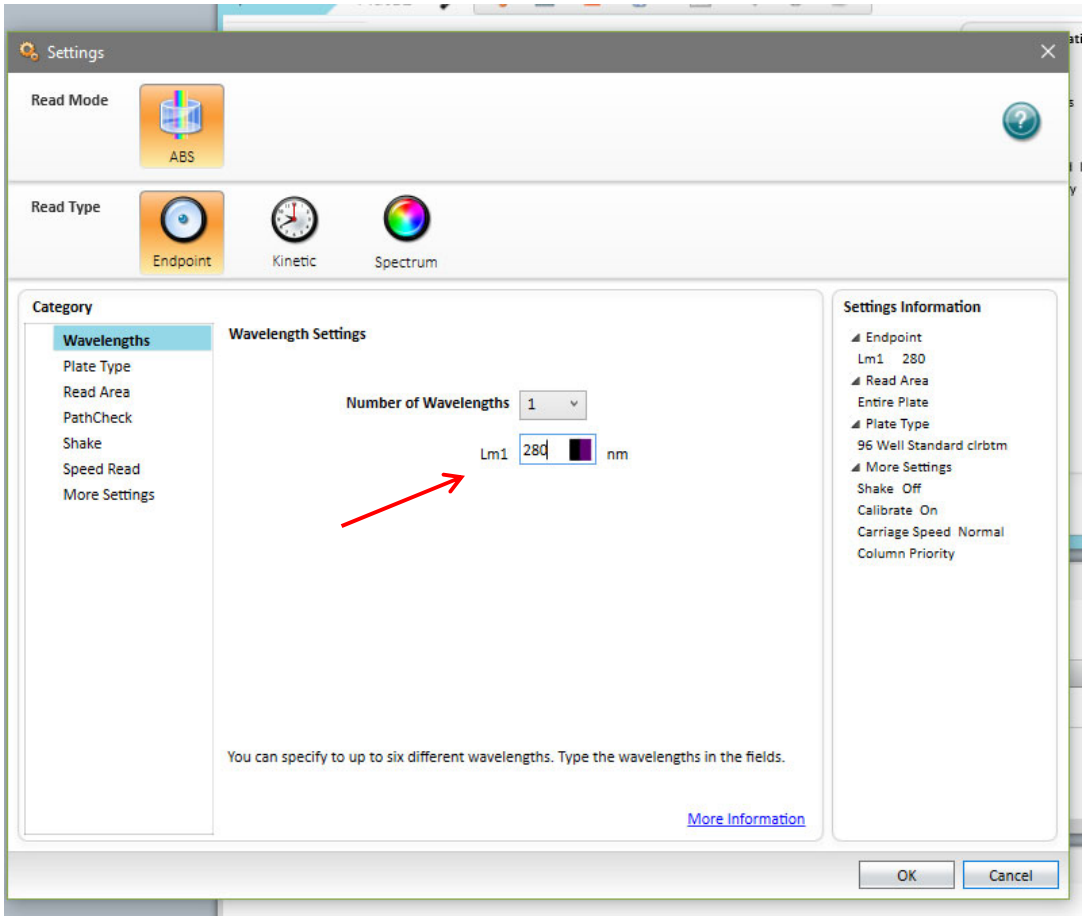

Figure 2: Acquisition setting window

- open the cuvette chamber
- place cuvette in the cuvette cavity (figure 3)

direction of the monochromatic light

Figure 3: Orientation of the cuvette window according to the direction of the monochromatic light (see arrow)

NOTE: The light goes from the back to the front, so the light window of the cuvette must also be aligned accordingly (vertically). By using the cap with factor 10, 3 - 5 µL sample volume is needed. Using the cap with factor 50 a lower sample volume of 0.7 - 4 µL has to be used.

- add the appropriate volume of the corresponding blank solution (buffer reference) to the tray cell cuvette (figure 4)

Figure 4: tray cell cuvette

Figure 5: cuvette with cap

- put the cap on top of the tray (figure 5)
- press “Ref” in the top menu to set the blank (red arrow in figure 6)
- as an option press “Read” to measure the blank (blue arrow in figure 6)

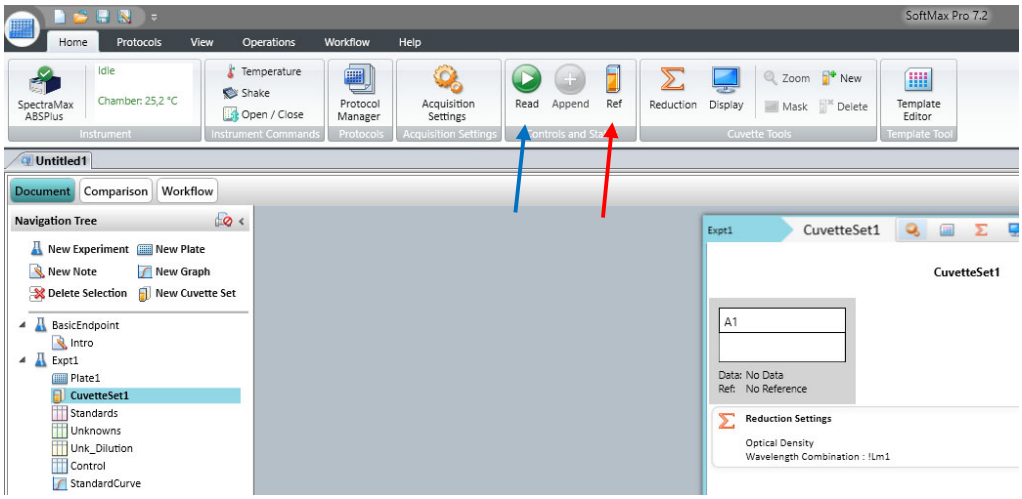

Figure 6: Reference measurement

- clean the tray cell and cap with a Kimtech wipe
- add same volume as for the blank to the tray cell
- place the cap on top
- press “Read” to measure the sample

NOTE: If “factor 10” is indicated on cap, the OD results have to be multiplied by 10 before further calculation; the same procedure applies when using the “factor 50” cap.

- clean the tray cell and cuvette cap with Kimtech and continue the measurements
- finally, tray cell and cuvette cap have to be cleaned with Kimtech wipe and 70% Ethanol
- close the cuvette chamber lid when measurements are finished

5.2 Measurement of a 96-well plate

5.2.1 Select an assay protocol

The default setup of the SoftMax Pro 7.2. software is a 96 well plate (96 WP) format. The software contains various ready to use protocols (see figure 7).

- click on “Protocols” in the main menu and navigate to “Protocol Manager” → “Protocol Library”, select an application field and choose a specific assay (see figure 8)

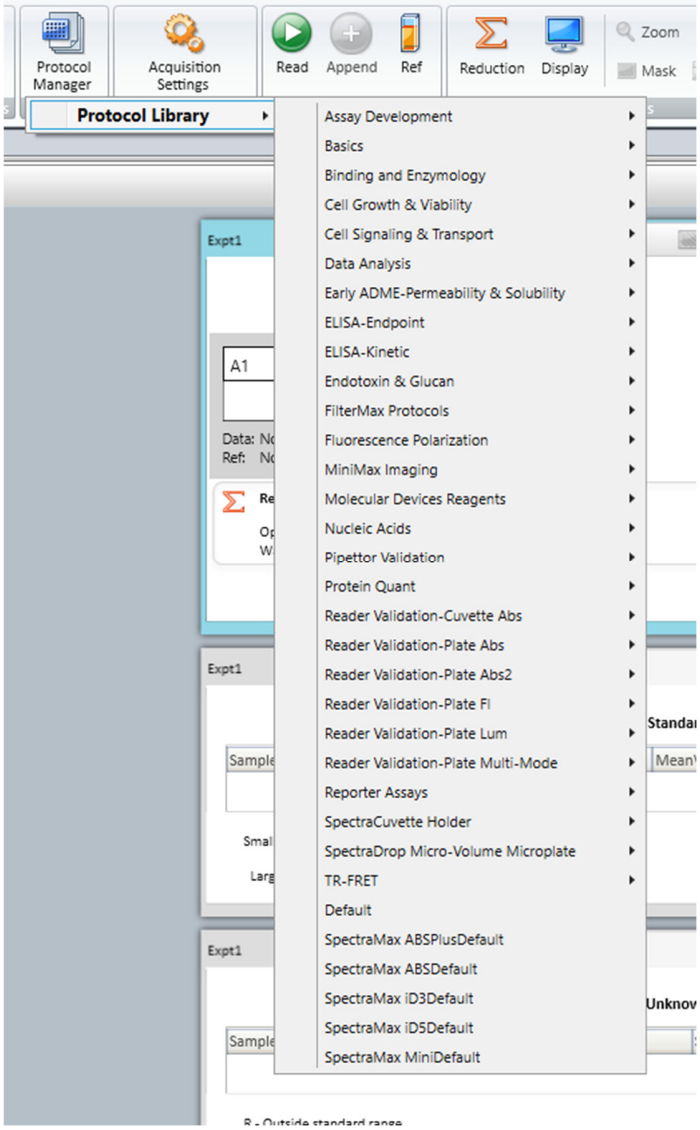

Figure 7: Overview of available application protocols in SoftMax 7.2 software

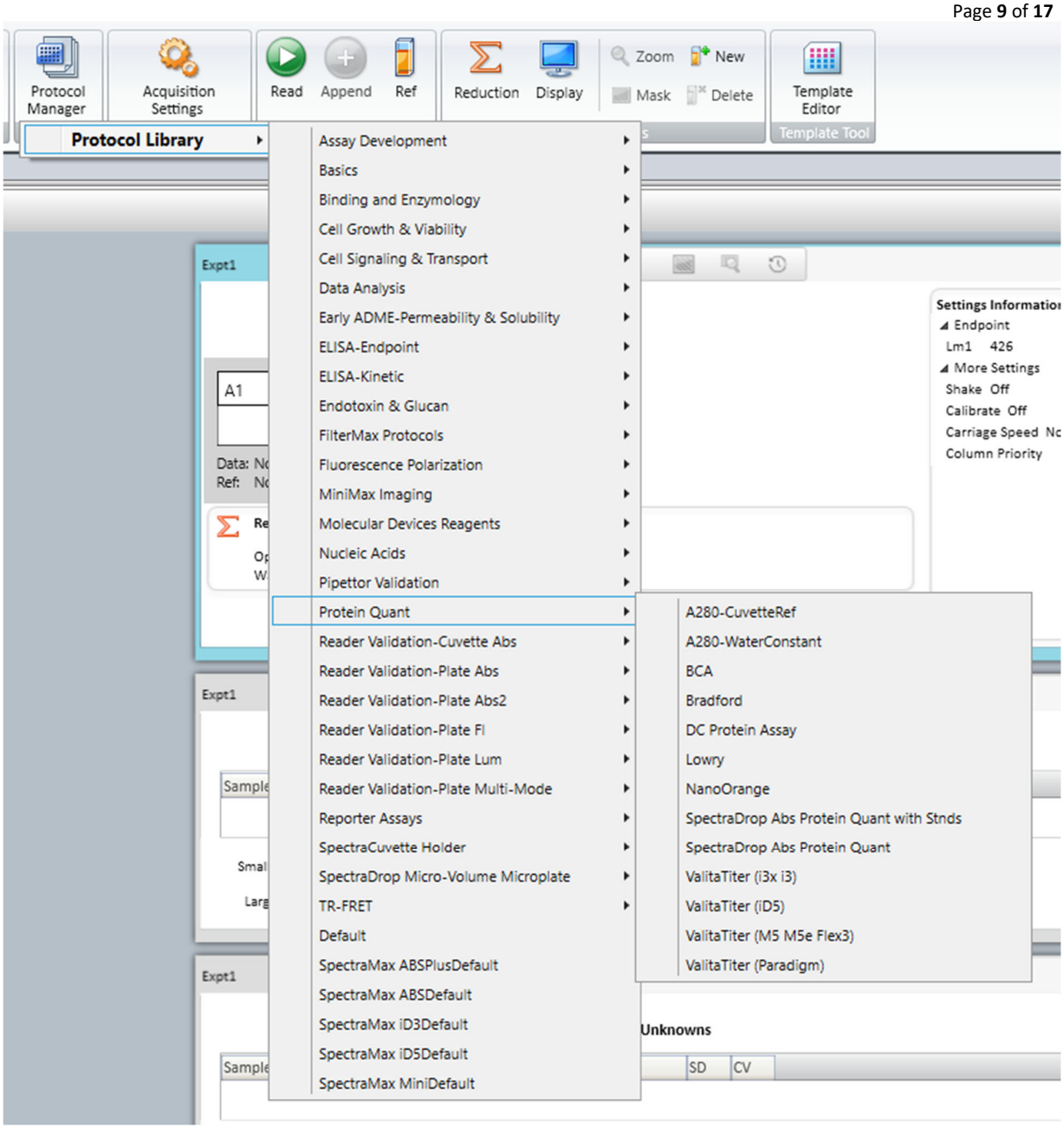

Figure 8: Example of ready to use 96 WP assays in the field of protein quantification

5.2.2 Definition of an assay template

- before starting a measurement, define the plate layout (wells with blank, standards, samples) by pressing the “Template Editor” button (see arrow in figure 9)

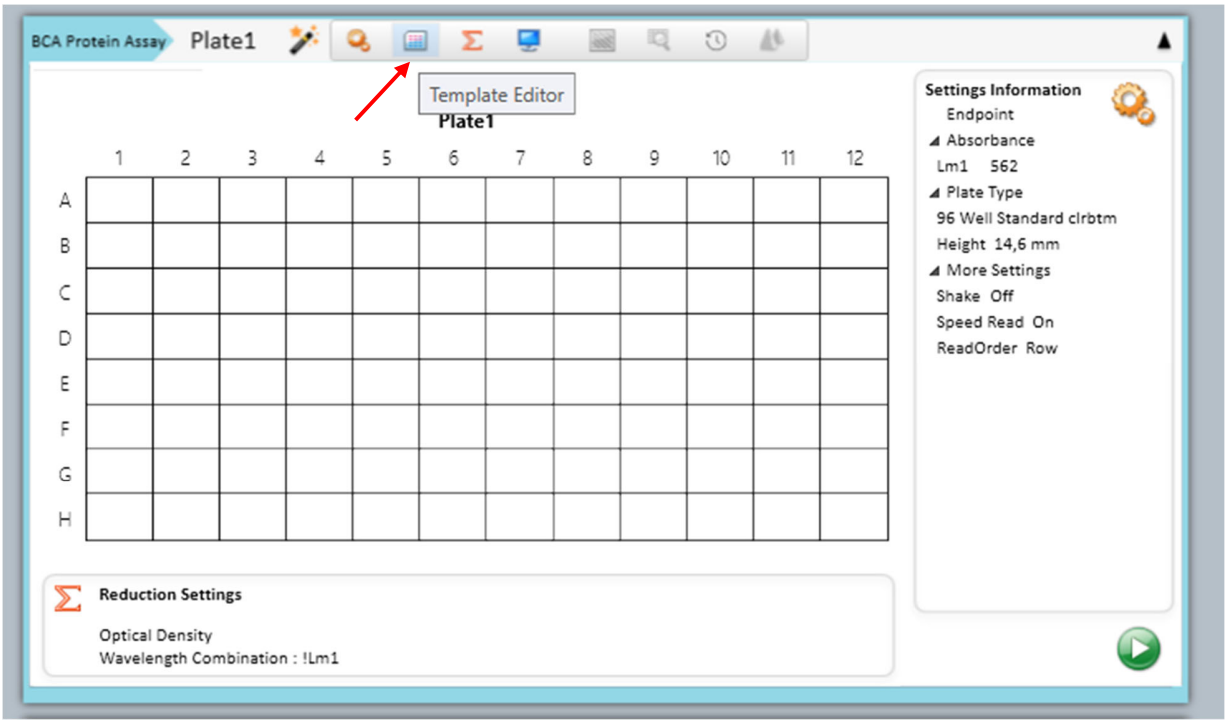

Figure 9: Default screen, start of the template editor

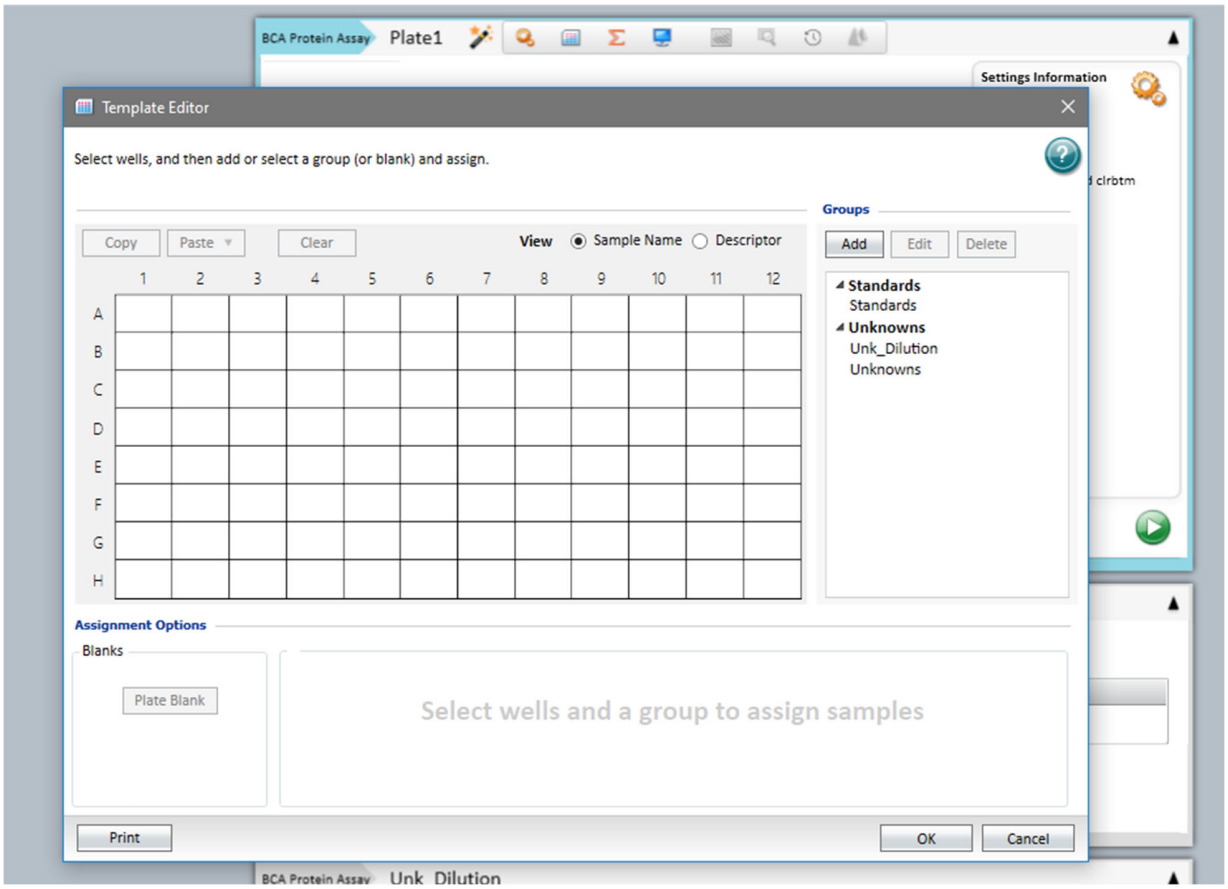

Figure 10: Template Editor start screen

- select wells to define a standard dilution series
- click “standard” in the right menu (see figure 11)

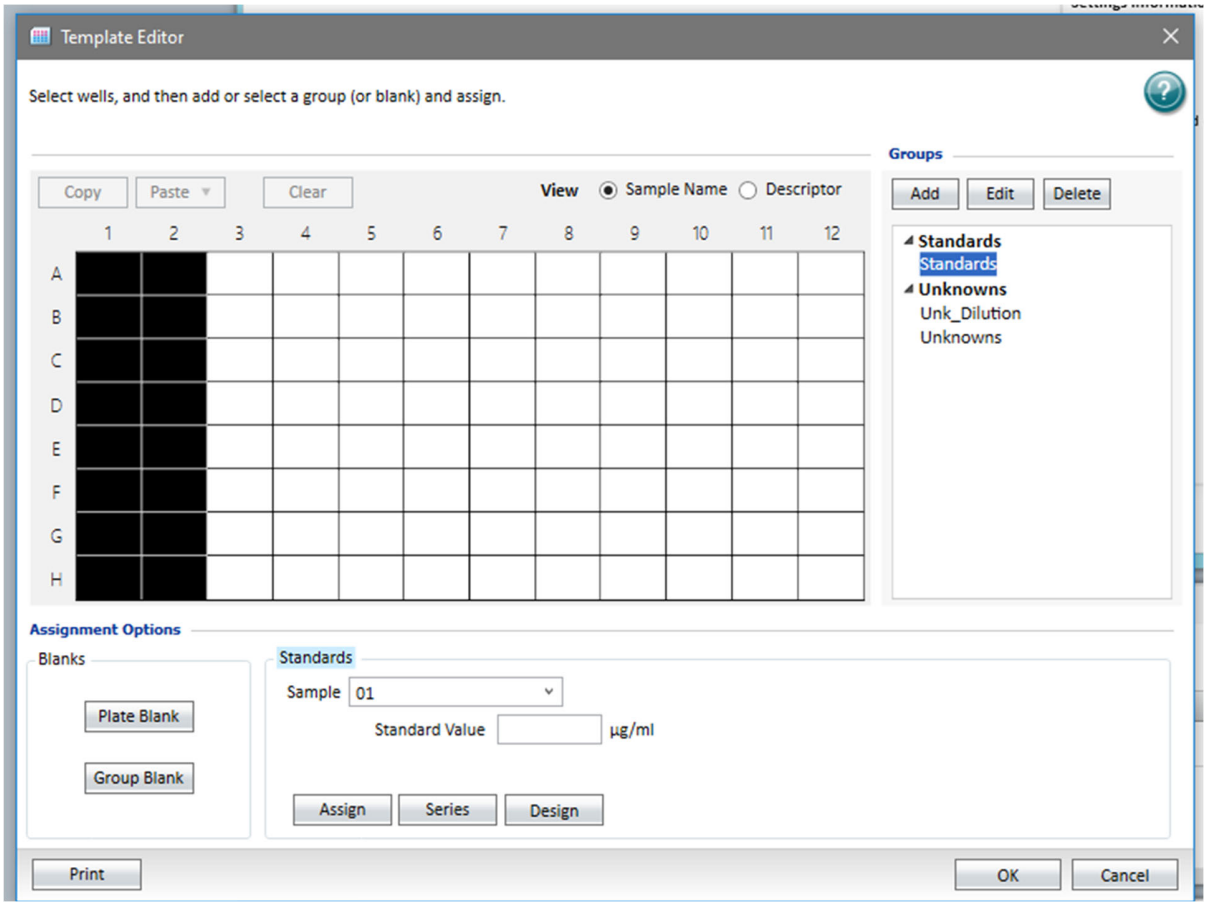

Figure 11: Options to define a standard curve in the template editor

NOTE: You can assign standard replicates individually by assigning a concentration to each standard dilution or you can define a series.

- click on the “Series” button to define a standard series
- a new window will open (see figure 12)
- enter the name, the start concentration and the dilution factor of your standard series
- adjust the pattern of the standard replicates if necessary
- confirm the settings by clicking the “ok” button

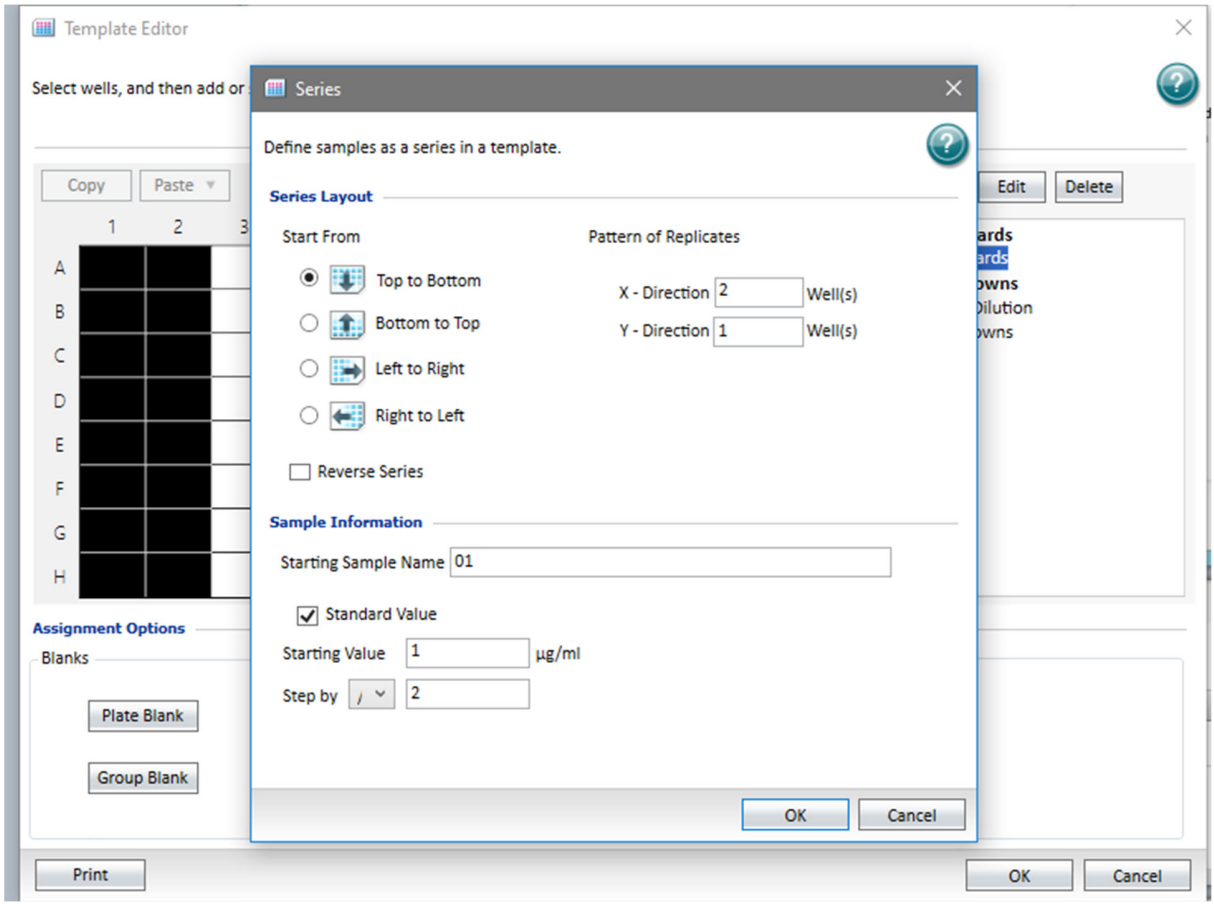

Figure 12: Options to define a serial dilution standard

NOTE: The sample replicates can be defined separately or as a series similar to a standard dilution series.

- select wells to define the samples / sample dilution series
- click “unknown” in the right menu (see figure 13)
- define sample names and location and number of replicates
- confirm with “ok”

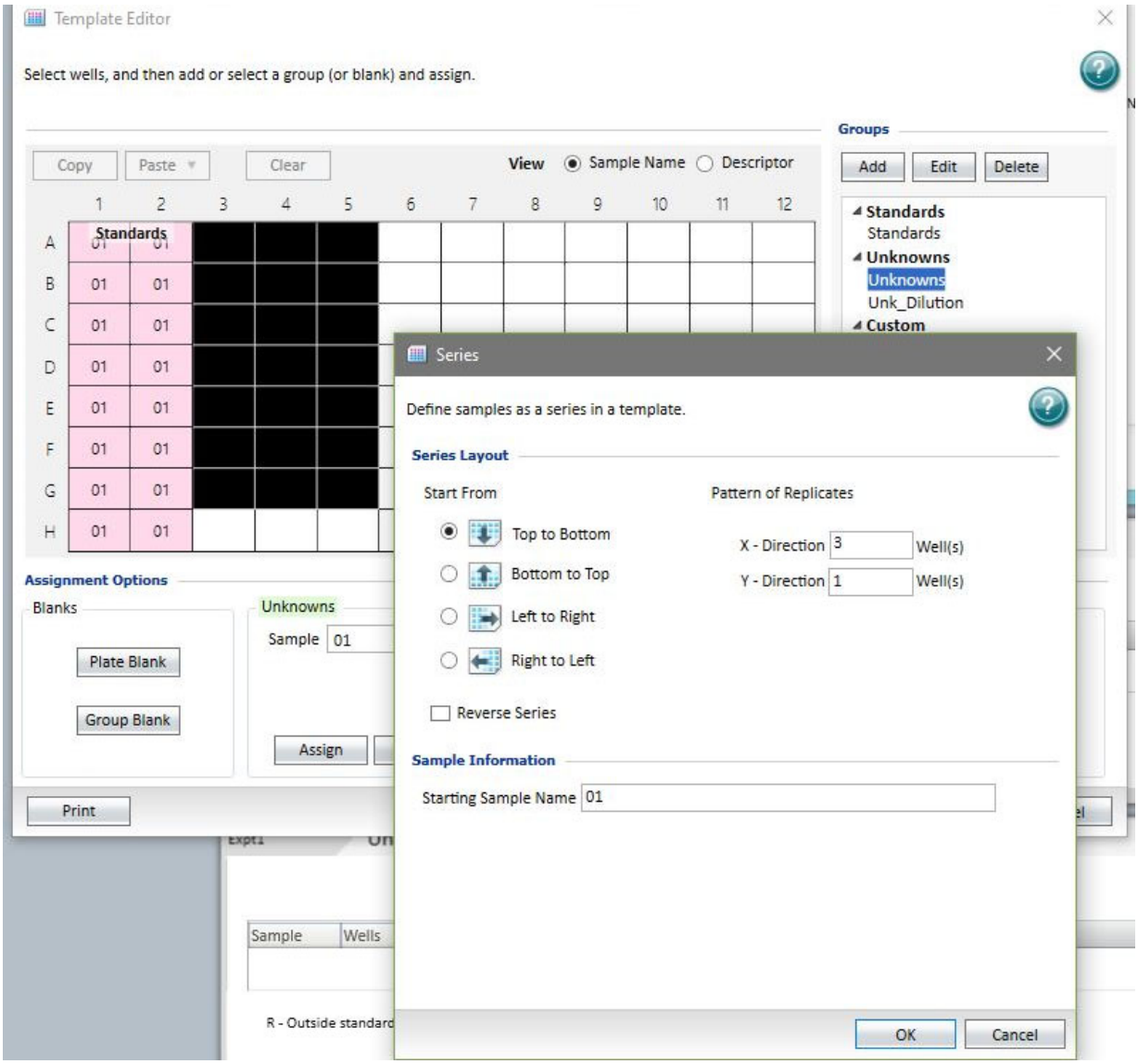

Figure 13: Options to define a sample series

- when the template is completely set, click on “OK”
- define an assay temperature if necessary (see chapter 5.1.3)
- place your plate in the drawer, press “open/close” button (instrument commands) to return the drawer into the photometer and press “Read”

5.2.3 Temperature definition and mixing

- in the top menu “Instrument Controls” you can define a temperature (+5 – 45°C) for your measurement
- click on the ”on” button and define a temperature (figure 14)

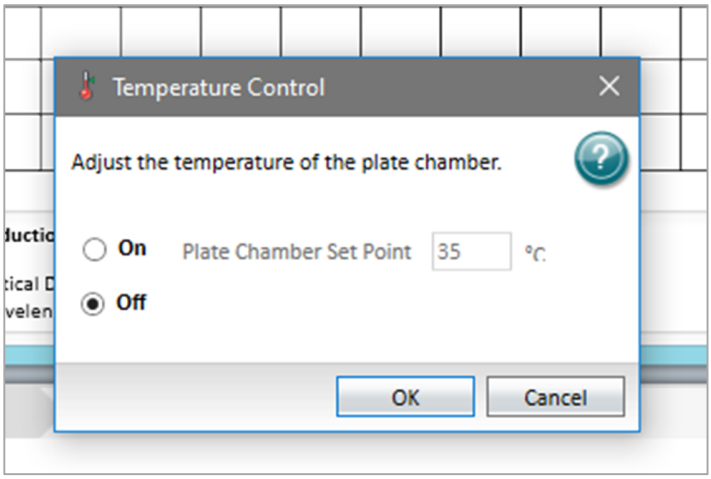

Figure 14: Temperature control setting options (range: +5°C – 45°C)

- a plate can be mixed by pressing the “shake” button
- after the measurement the results can be printed, saved or exported

5.3 Saving and exporting data

- click on the 96WP Icon in the upper left corner to open the save and export options (see figure 15)
- data can be saved as data files (*.sda) or as protocol files (*.spr)
- data can be exported as text files (*.txt), Excel file (*.xls) or as XML file (*.xml)

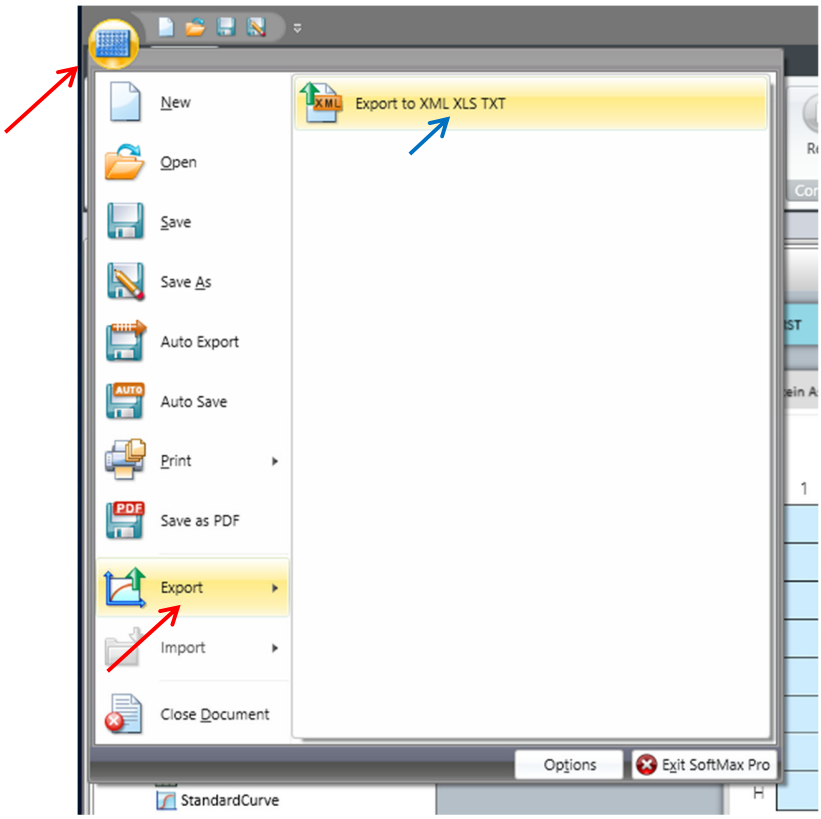

Figure 15: Saving and exportating data

- select a folder and save your data
- remove your plate and close the drawer

5.4 Switch plate format to 384 well plate

- go to “Acquisition Settings”
- go to “Plate Type” and choose in the dropdown menu of the “Plate Format” “384 wells” (figure 16)

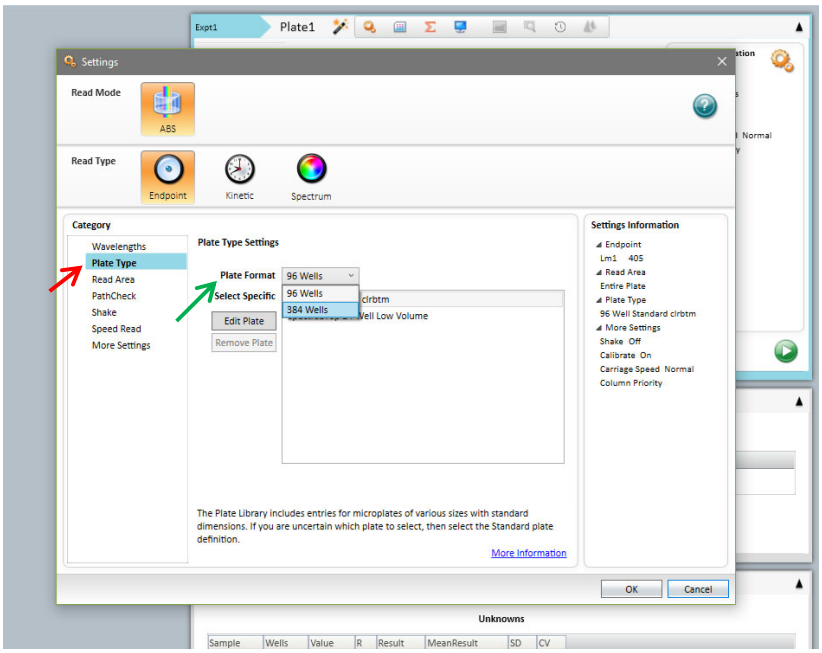

Figure 15: Saving and exportating data

6. Reference

Y:\Labmanager\Bestand\Geräte\Plate Reader\Bedienungsanleitungen\Spectramax ABS Plus\softmax-pro-user-guide_5305581a.pdf

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7. SOPs

A003 BCA Protein Assay