全光譜分析儀Multiskan GO 的技術原理及其在生命科學研究的應用

Product Specialist – Anthony Mui

岑祥股份有限公司
TSENG HSIANG LIFE SCIENCE LTD.

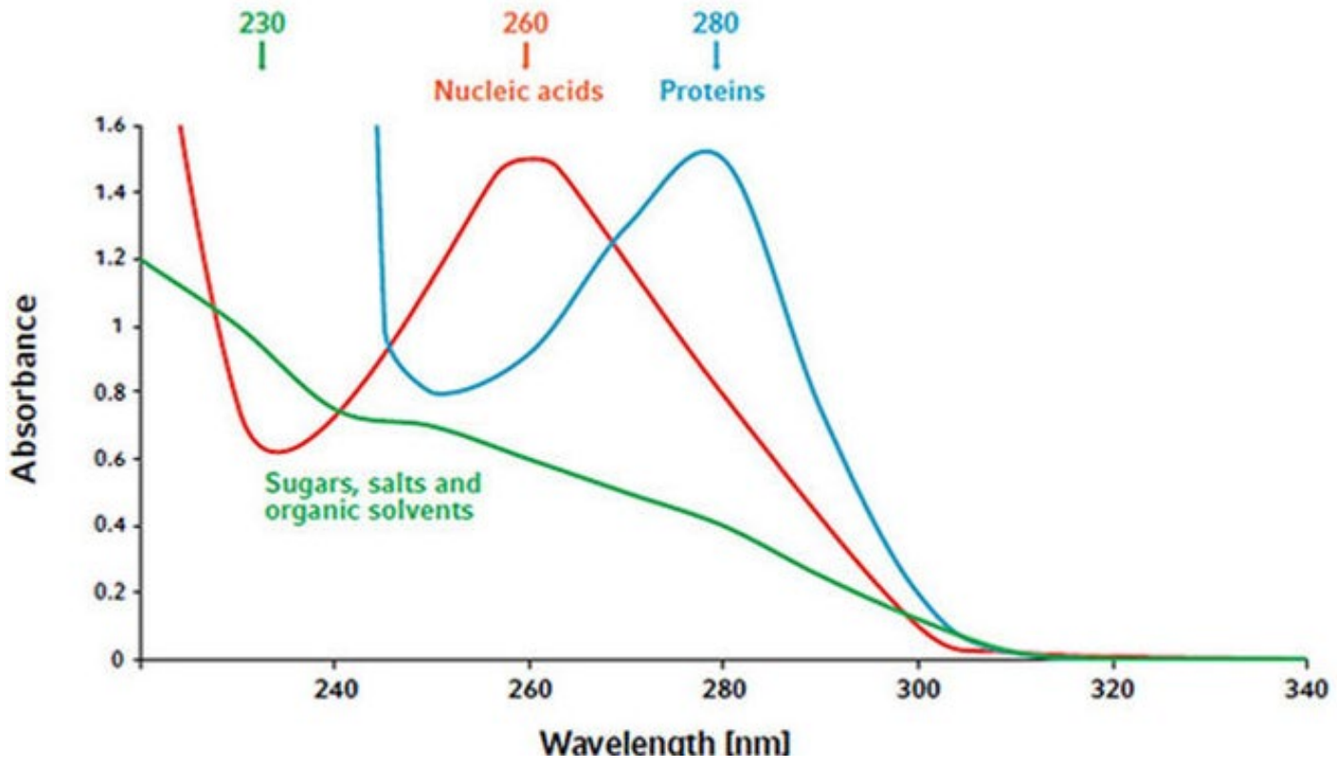
Overview

- Absorbance
- Application
- Introduction of Multiskan GO
- Operation

Absorbance

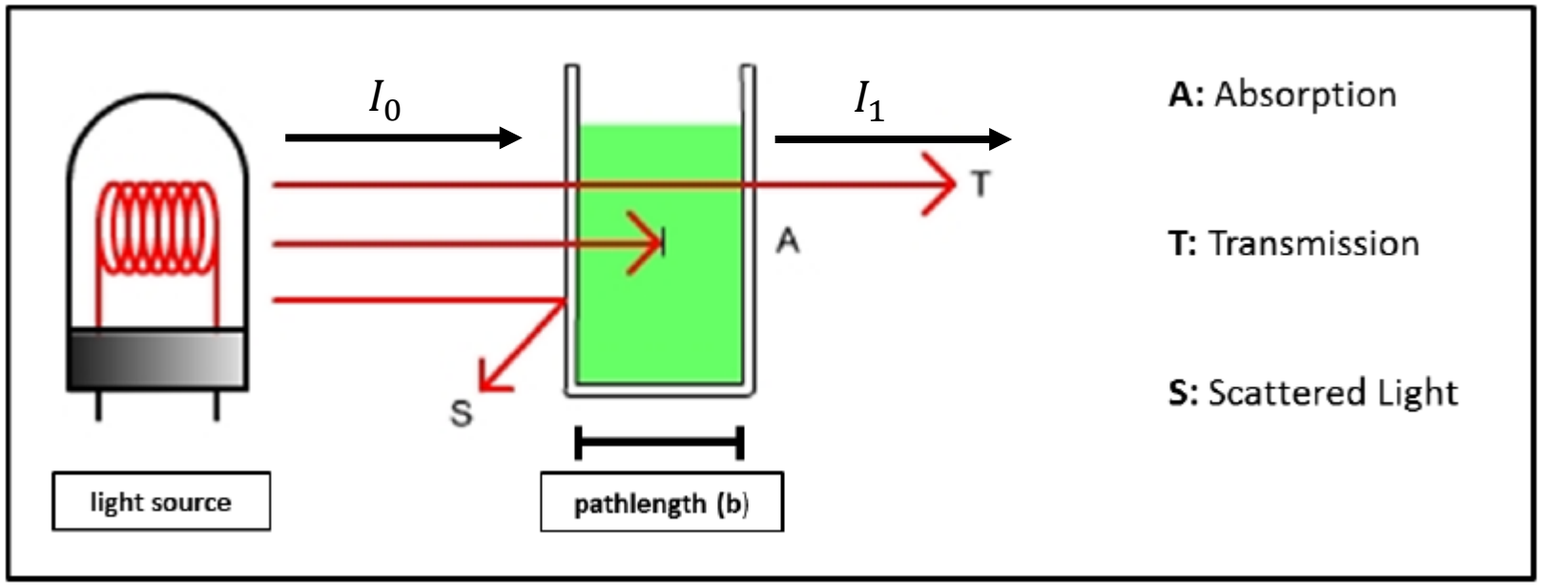
Why measure absorbance ?

In biology and chemistry, the principle of absorbance is used to **quantify absorbing molecules in solution**.



https://handling-solutions.eppendorf.com/sample-handling/photometry/applications/detailview-applications/news/uv-vis-spectrophotometry-easy-and-quick-quantification-of-nucleic-acids/

Absorbance



$$Transmittance[T] = \frac{I_1}{I_o}$$

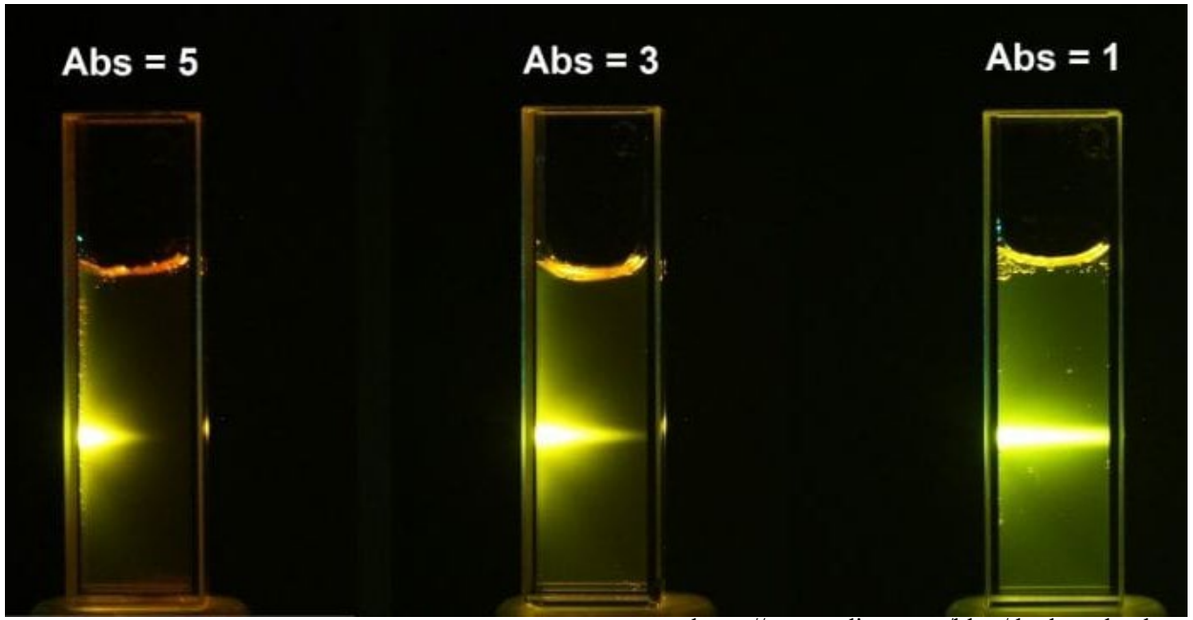
$$Absorbance[A] = -\log_{10}\left[\frac{I_0}{I_1}\right] = -\log_{10}[\frac{1}{T}]$$

I_1 = intensity of the radiation (light)

I_0 = amount of light of the incident light beam

Absorbance

Absorbance[A][O.D.]	Transmittance[T%]
0	100%
1	10%
2	1%
3	0.1%
4	0.01%
5	0.001%



https://www.edinst.com/blog/the-beer-lambert-law/

Figure : Attenuation of a 510 nm laser through three solutions of Rhodamine 6G with different absorbance values at 510 nm. The yellow glow is the fluorescence emission at ~560 nm

That means a sample with:

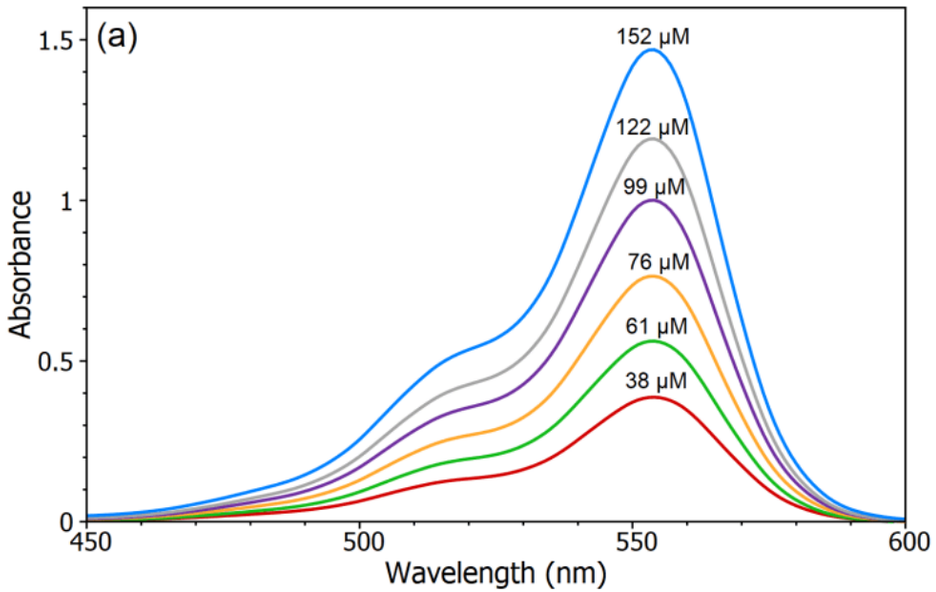
- 1 O.D. allows 10% of light to be transmitted through the sample
- 2 O.D. allows 1% of light to be transmitted through the sample
- 3 O.D. allows 0.1% of light to be transmitted through the sample
- 4 O.D. allows 0.01% of light to be transmitted through the sample

Absorbance

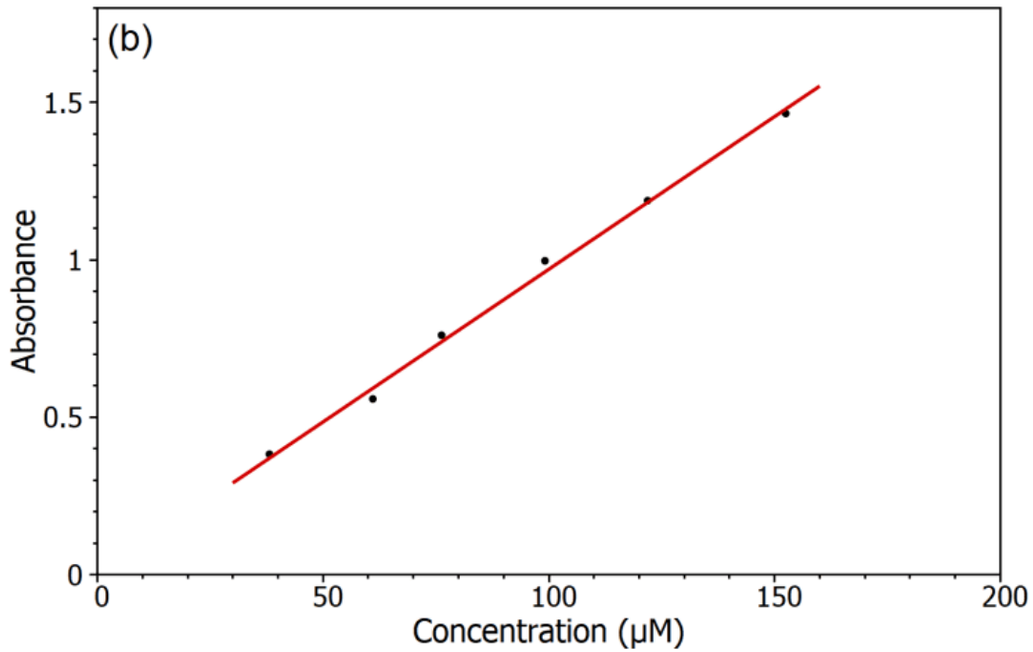
The Beer's law

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm



Standard curve



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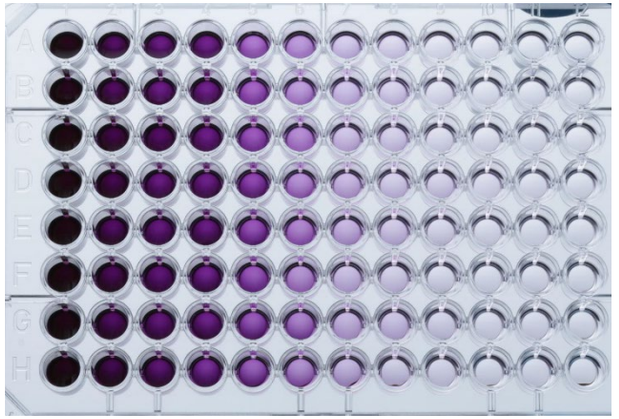
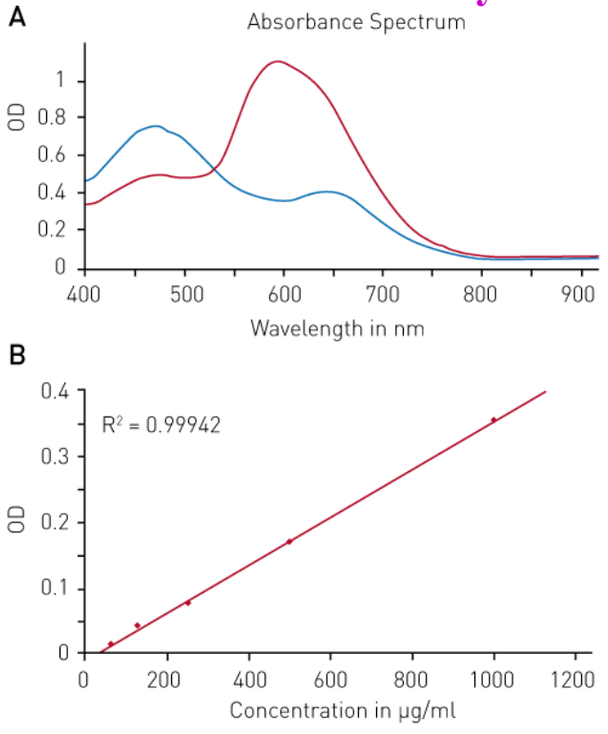
- ELISA
- Cell viability(MTT)
- BCA/Brafford
- DNA/Protein (260 nm / 280 nm)
- Microbial growth (OD 600 nm)

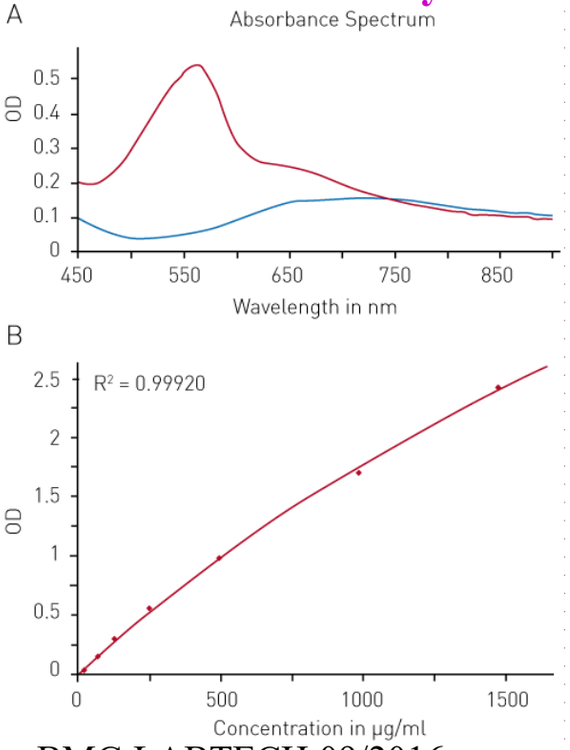


Cell viability(MTT Assay)

Bradford assay



BCA assay



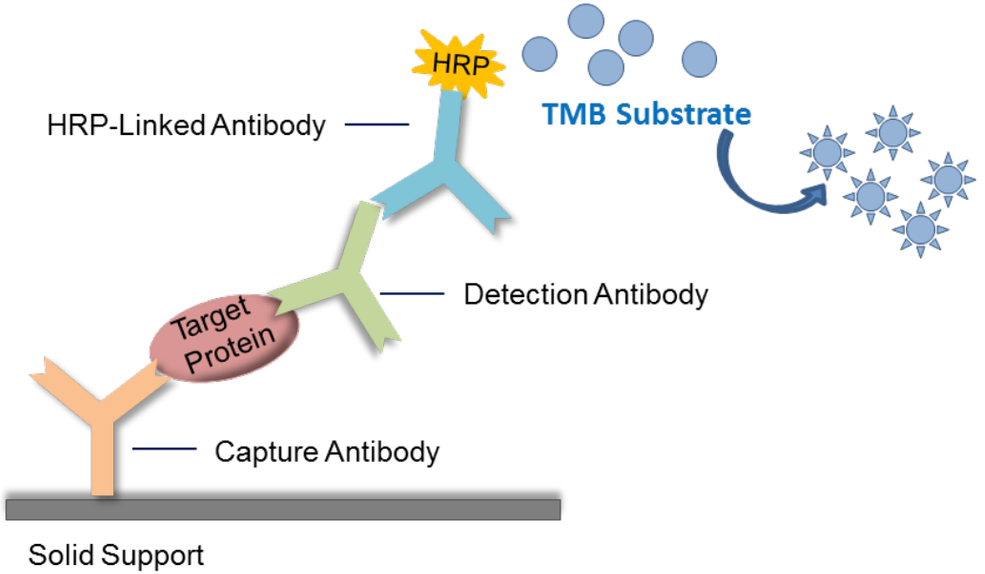
Andrea Krumm BMG LABTECH 09/2016

Application

ELISA

To detect specific protein by antibody

Usually measure 450 or 405 nm



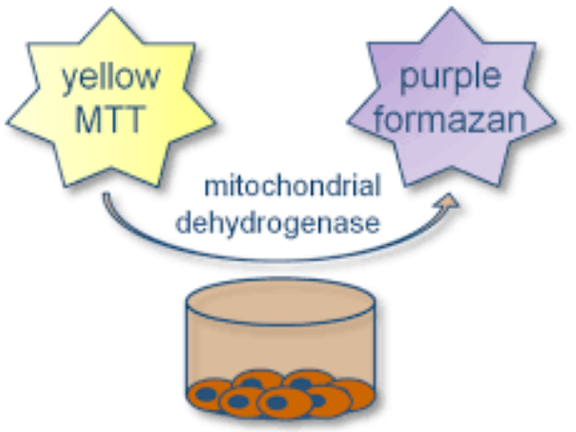
Cell viability CCK-8 AND MTT

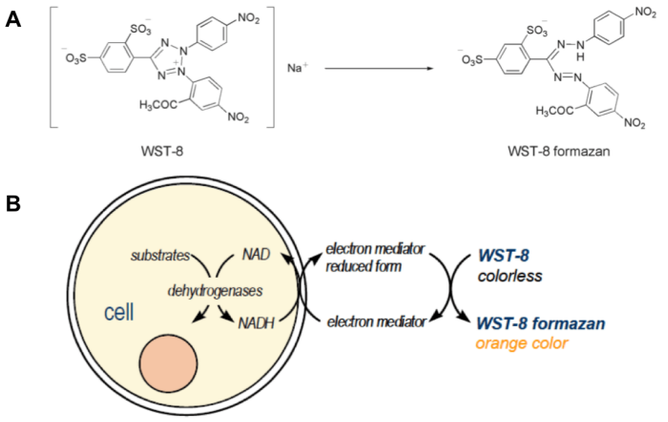
To check the vial cell number by specific reagent

Usually measure 450 and 570 nm

MTT = 570nm

CCK-8 = 450nm



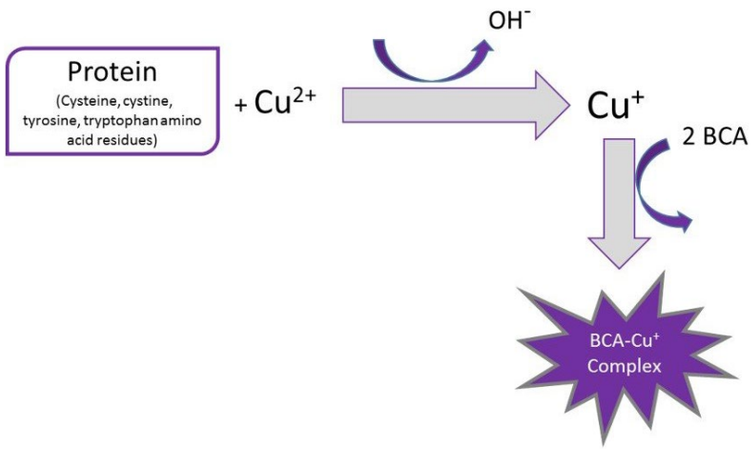


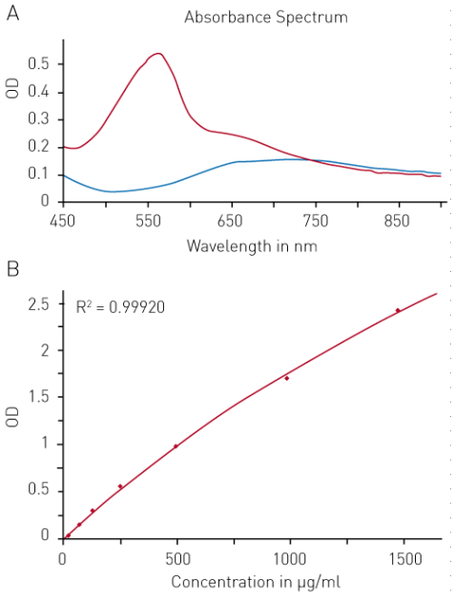
Application

BCA

To quantify protein in unknown sample

Usually measure 562 nm

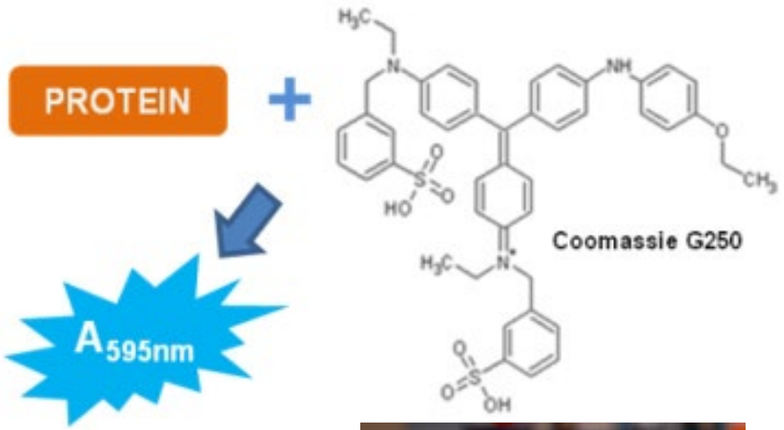


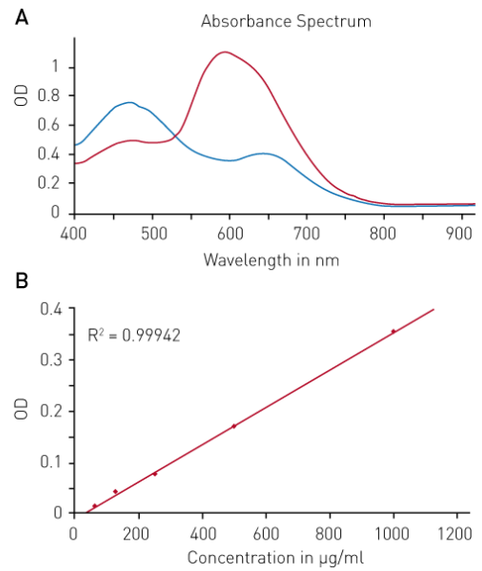


Brafford

To quantify protein in unknown sample

Usually measure 595 nm



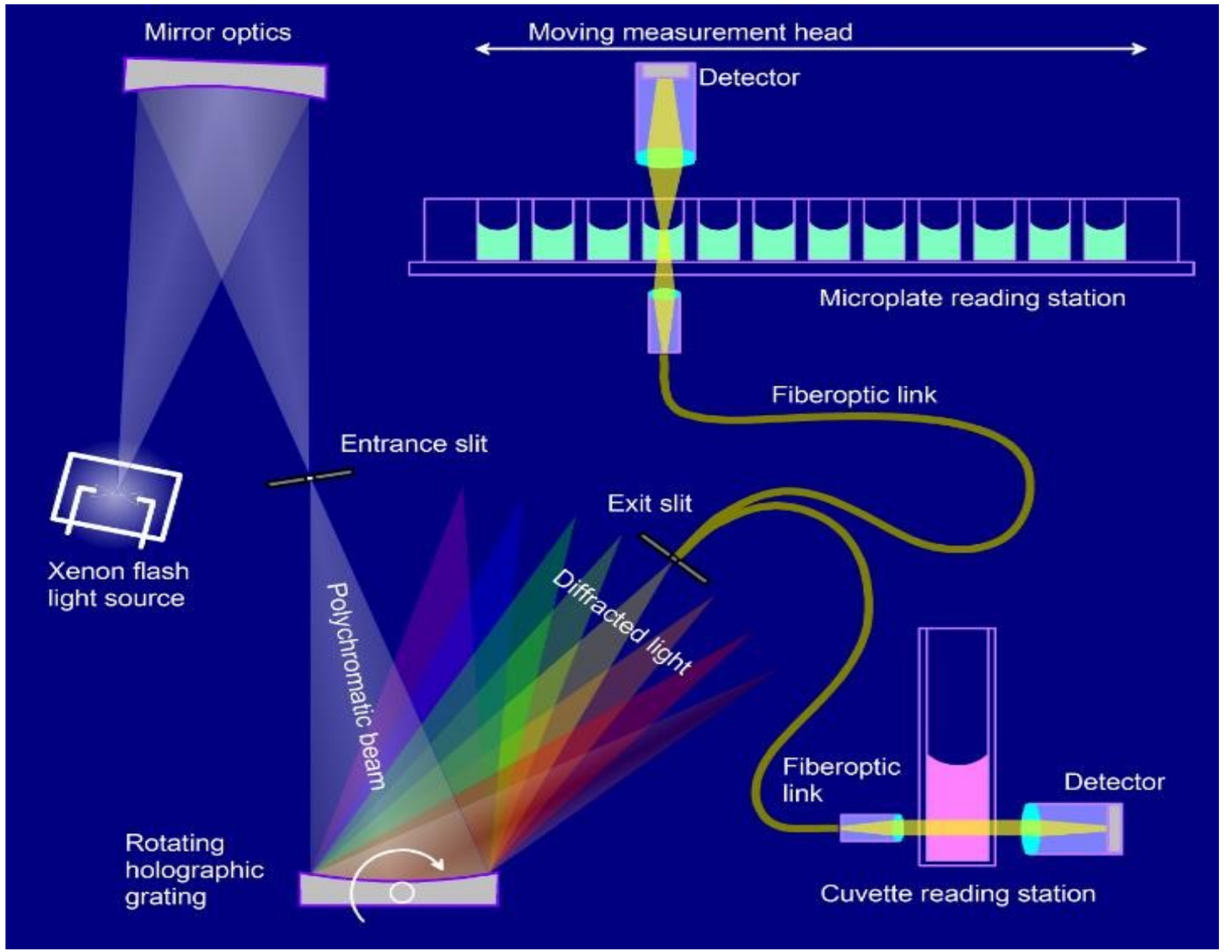


Introduction of Multiskan GO

	Multiskan GO
Wavelength	200 -1000 nm
Reads	96- and 384-well plates
Shaker	V
Incubation	up to +45°C
Reading speed	96-well plate in ~ 6 seconds
Spectrum	~10 seconds

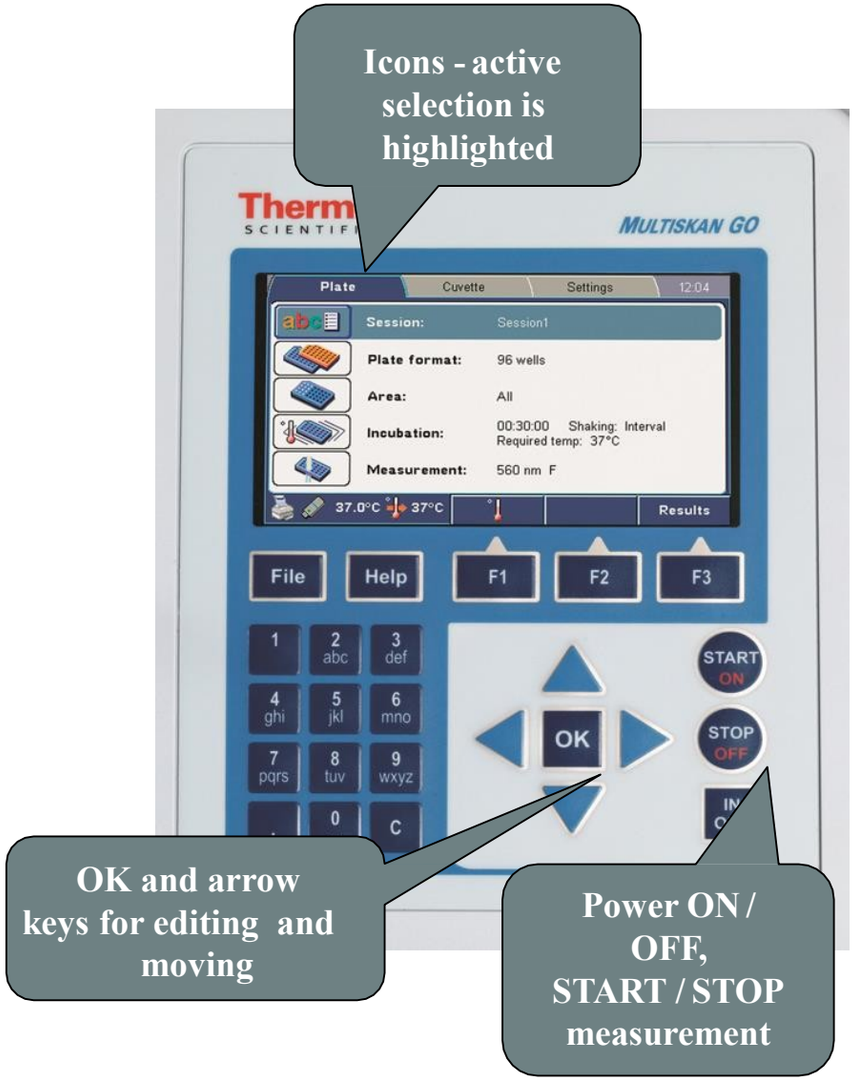
Introduction of Multiskan GO

Monochromator based system with adjustable wavelength

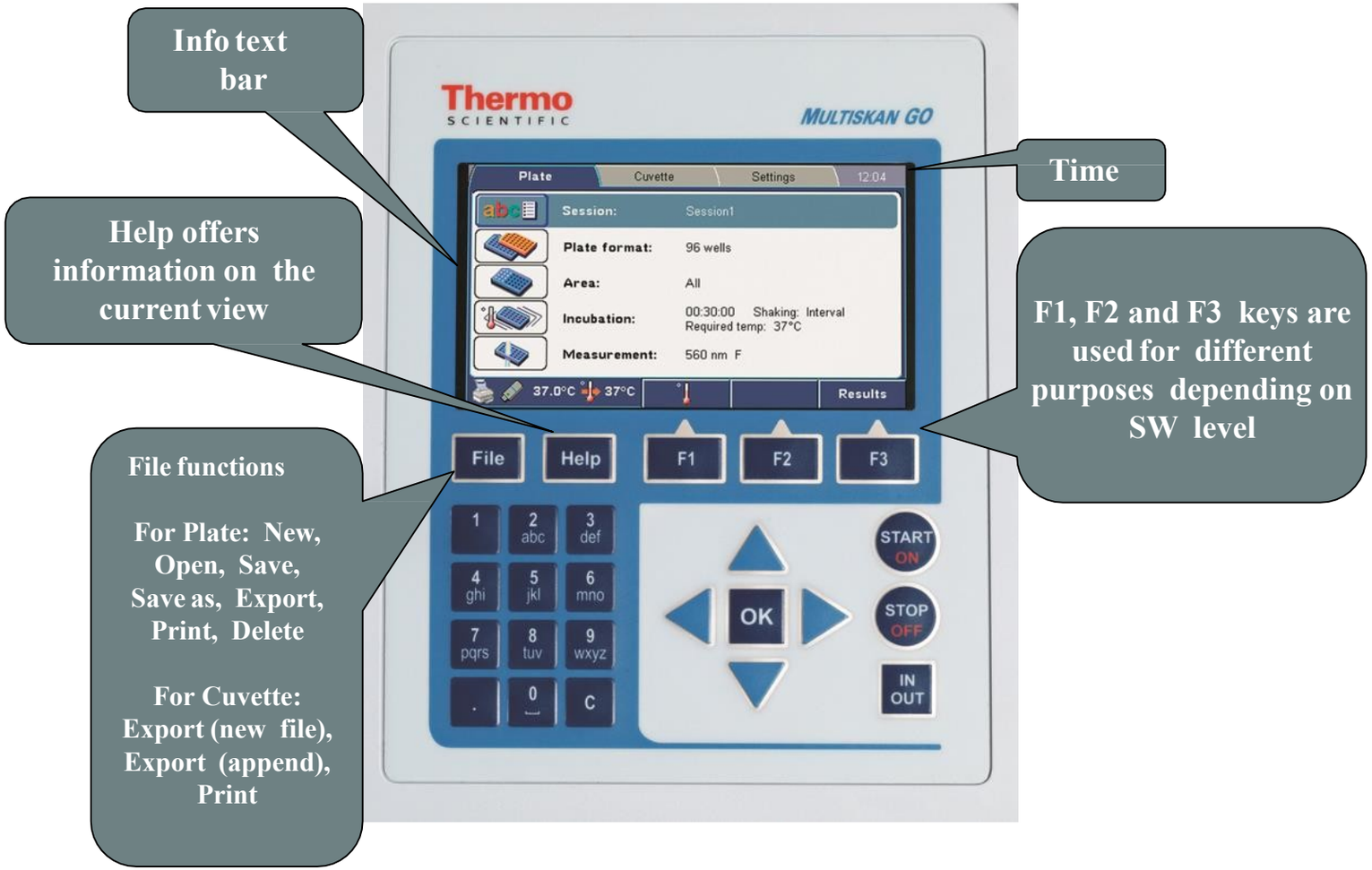


Operation

- User Interface tabs
  - **Plate**:
    defines protocol for the plate measurement
  - **Settings**:
    contains universal instrument
    configuration settings
    - Configuration, Date&Time, Print header, Status report

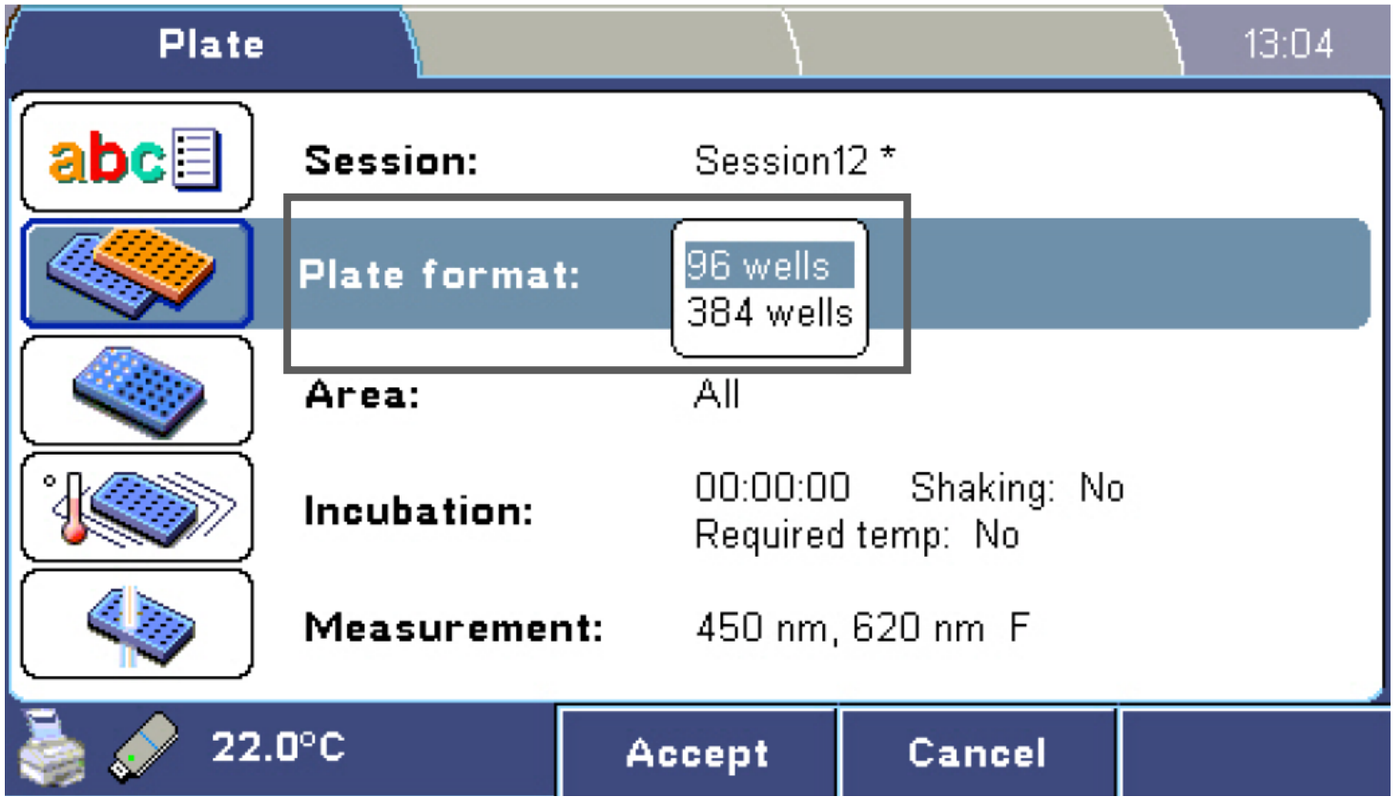


Operation


Info text bar
Help offers information on the current view
File functions
For Plate: New, Open, Save, Save as, Export, Print, Delete
For Cuvette: Export (new file), Export (append), Print
Time
F1, F2 and F3 keys are used for different purposes depending on SW level
Thermo SCIENTIFIC
MULTISKAN GO
Plate
Cuvette
Settings
12:04
Session:
Session1
Plate format:
96 wells
Area:
All
Incubation:
00:30:00 Shaking: Interval
Required temp: 37°C
Measurement:
560 nm F
37.0°C
37°C
Results
File
Help
F1
F2
F3
1
2 abc
3 def
4 ghi
5 jkl
6 mno
7 pqrs
8 tuv
9 wxyz
.
0
C
OK
START ON
STOP OFF
IN OUT

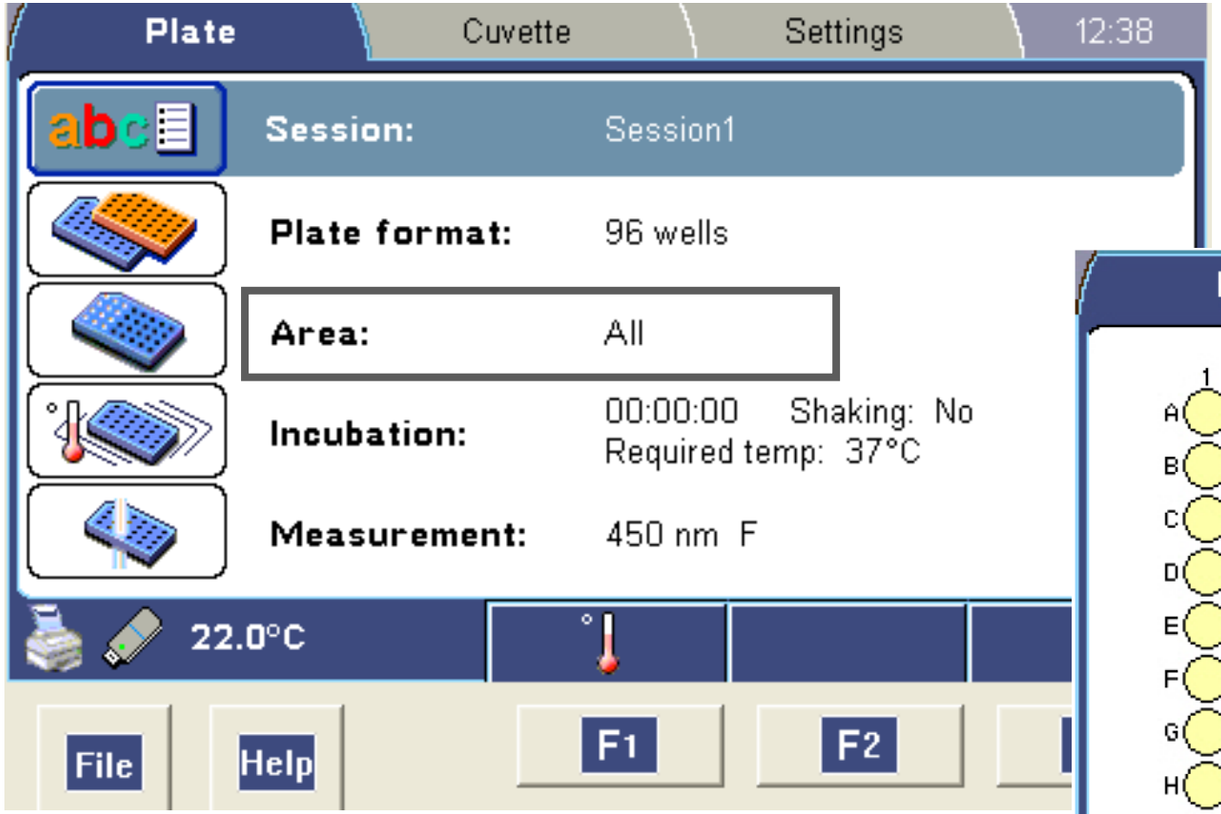
Operation-Plate measurement

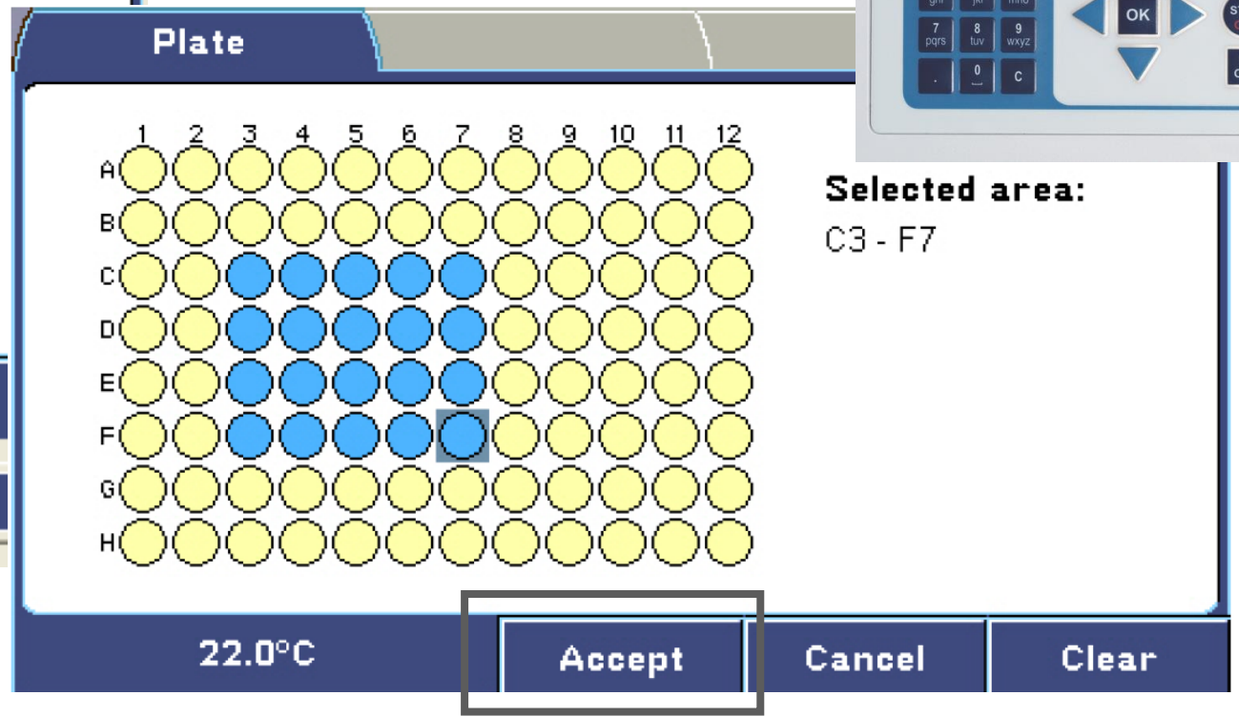
- **Selecting “Plate format”**



Operation-Plate measurement

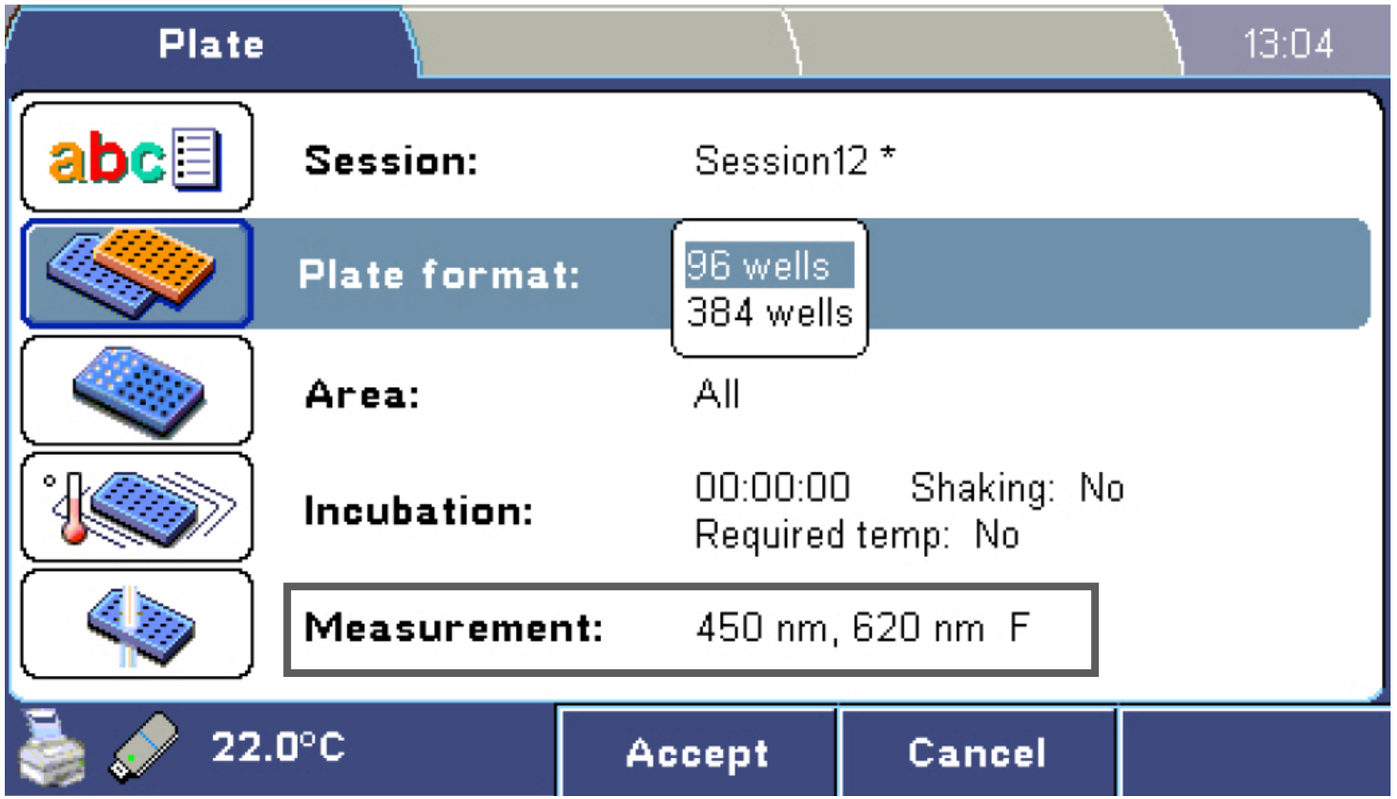
- **Selecting "Area"**





Operation-Plate measurement

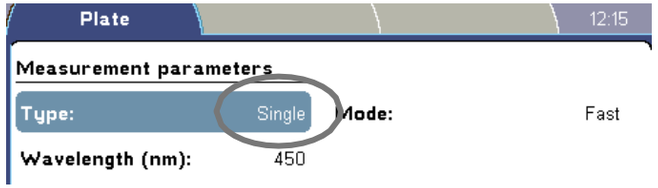
- Setting “Measurement"



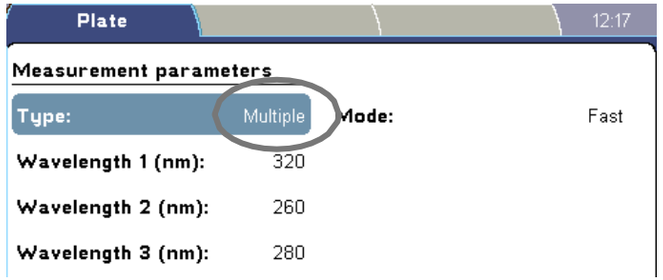
Operation-Plate measurement

Measurement types

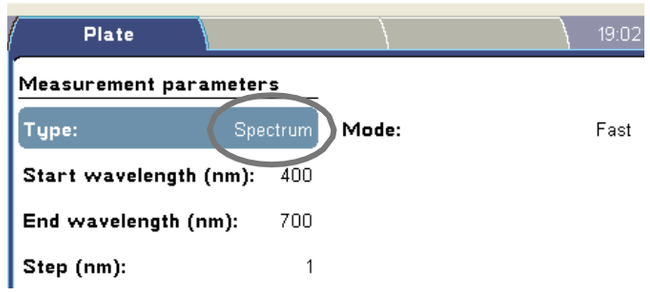
Single

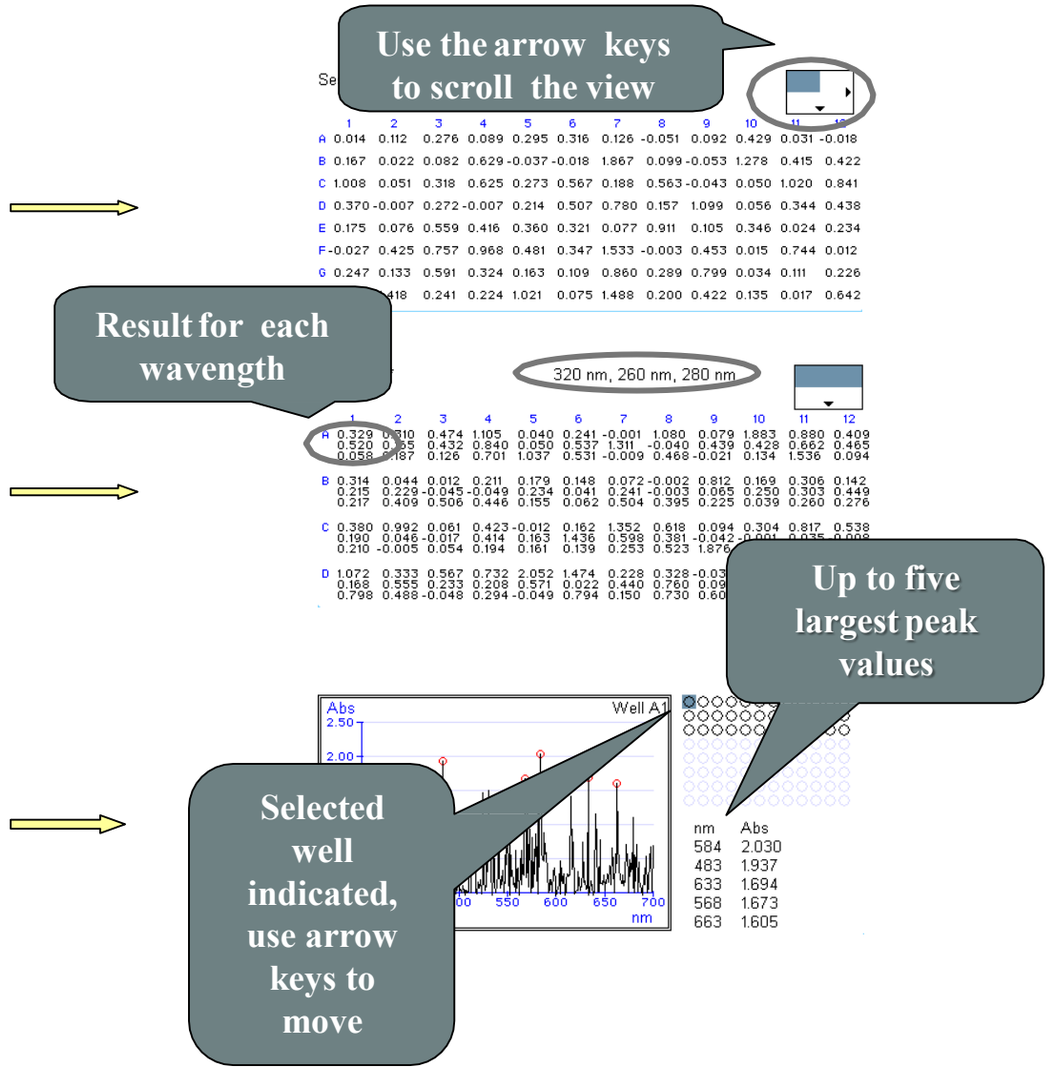


Multiple



Spectrum



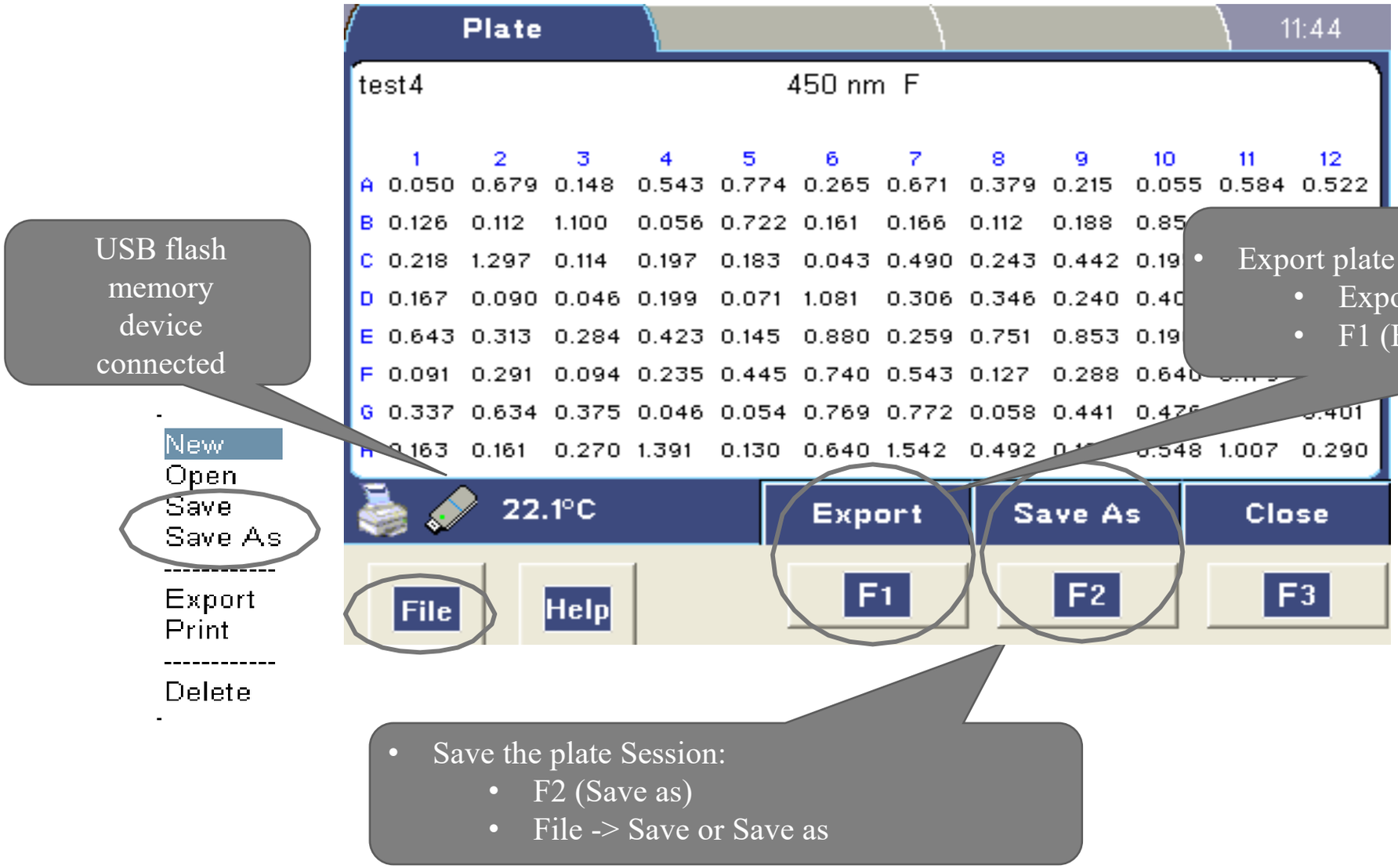


Operation-Plate measurement

- **“START“ Measurement**

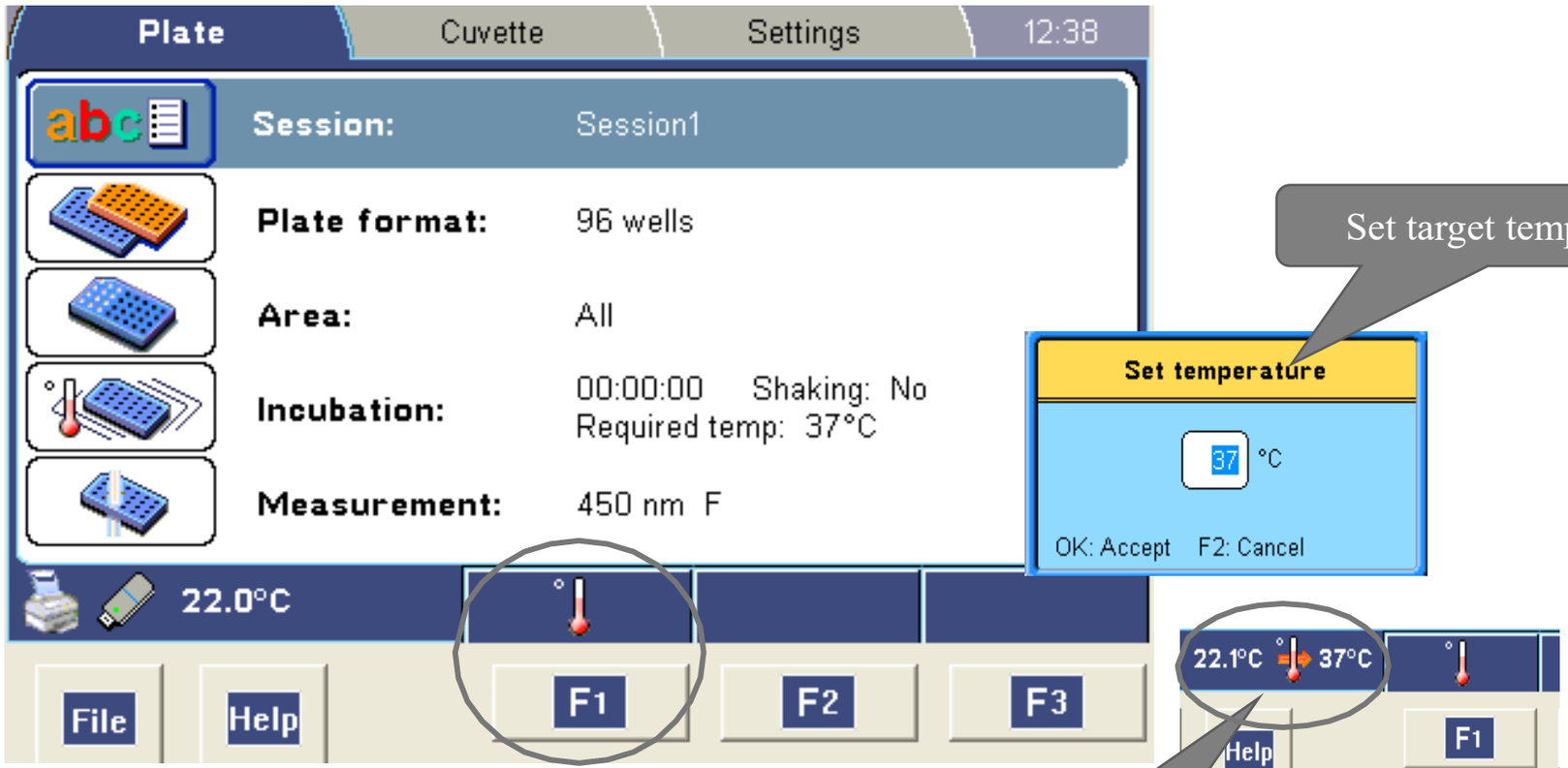
Operation-Plate measurement

- **"Saving" Result**



Operation-Incubator

- **“Incubation”**



Set target temperature

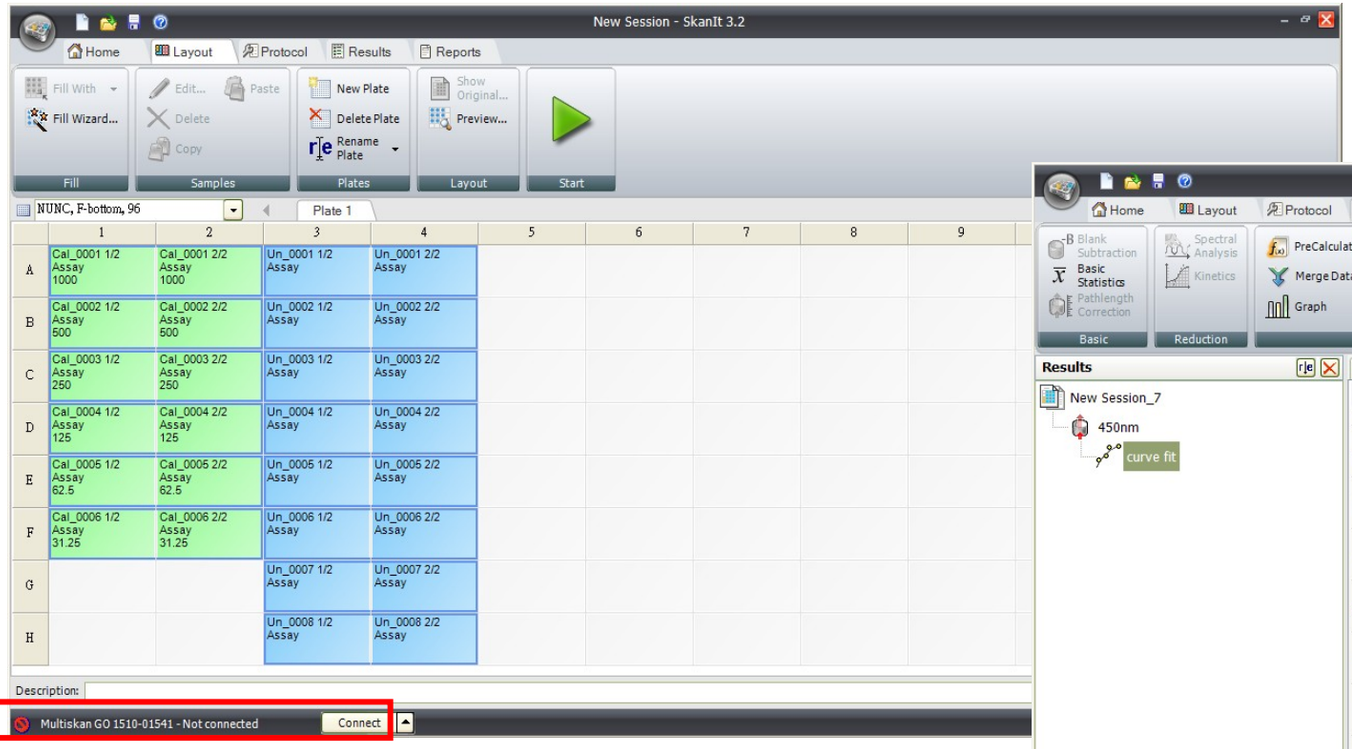
- The incubator temperature can be controlled using F1 key

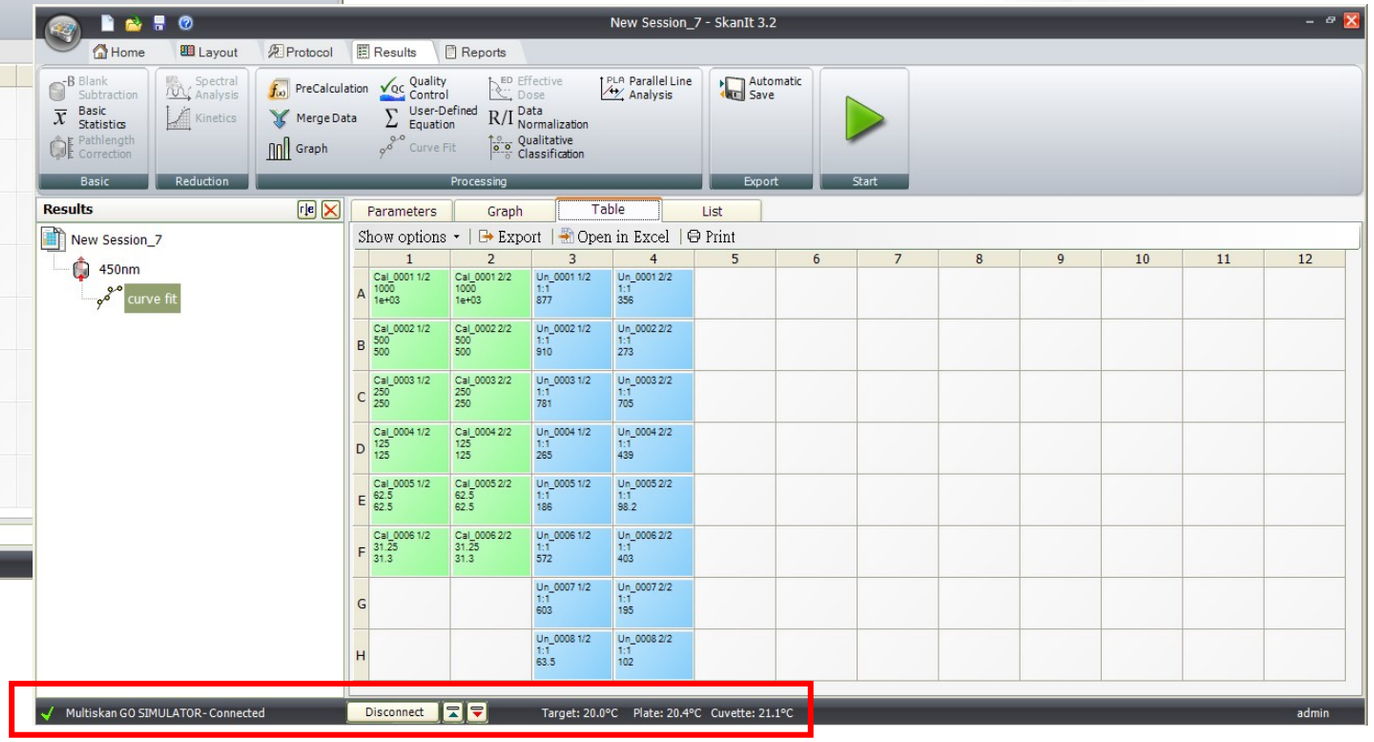
Instrument and Target temperature

Note!
The “Required temp” parameter” sets an incubation temperature reminder.
It does not affect the instrument temperature

Operation

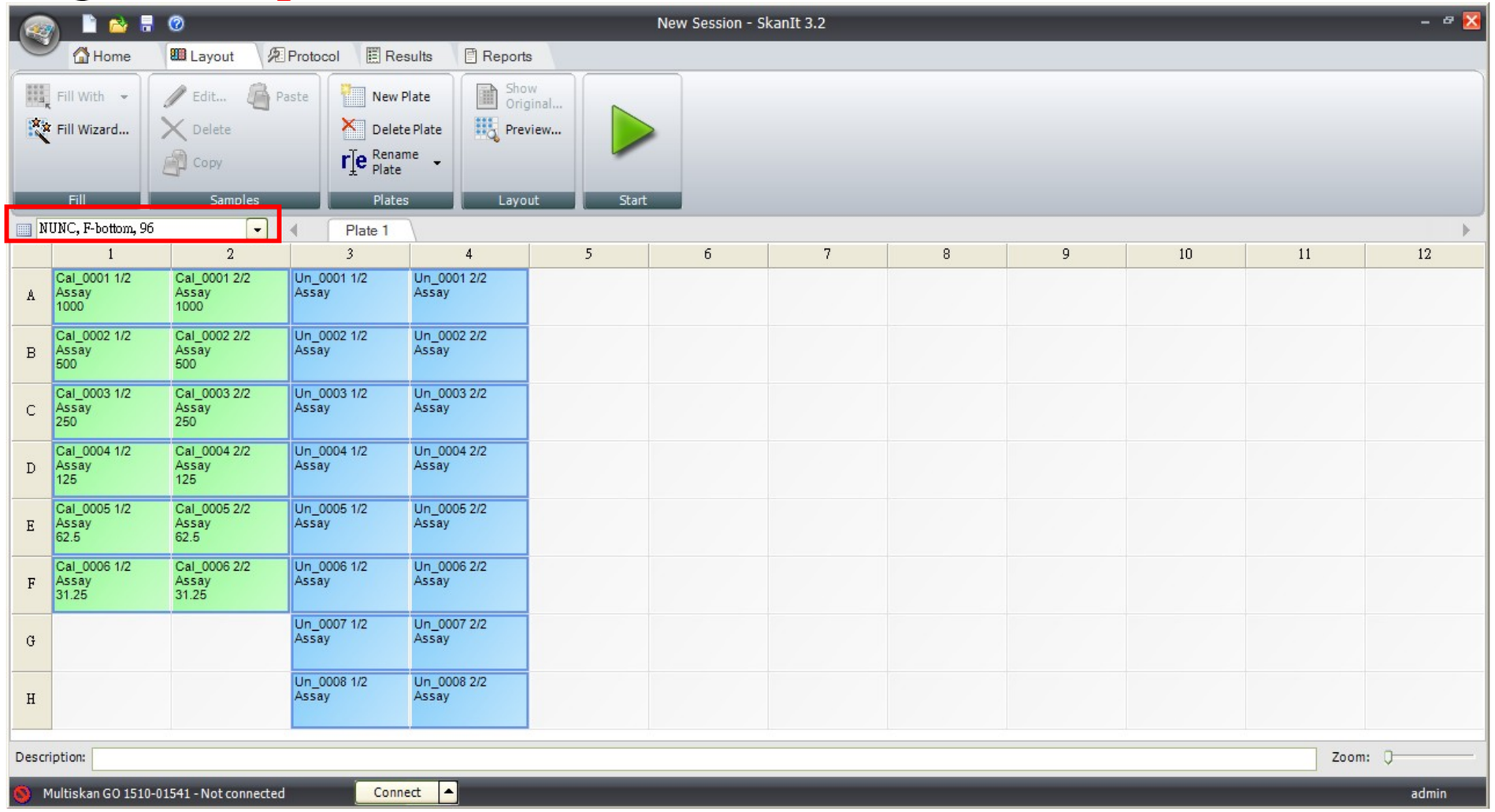

SkanIt
Software
thermo
scientific


New Session - SkanIt 3.2
Home
Layout
Protocol
Results
Reports
Fill With
Fill Wizard...
Edit...
Delete
Copy
Paste
New Plate
Delete Plate
Rename Plate
Show Original...
Preview...
Start
Fill
Samples
Plates
Layout
NUNC, F-bottom, 96
Plate 1
Description:
Multiskan GO 1510-01541 - Not connected
Connect


New Session_7 - SkanIt 3.2
Home
Layout
Protocol
Results
Reports
Blank Subtraction
Basic Statistics
Pathlength Correction
Spectral Analysis
Kinetics
PreCalculation
Merge Data
Graph
Quality Control
User-Defined Equation
Curve Fit
Effective Dose
Data Normalization
Qualitative Classification
Parallel Line Analysis
Automatic Save
Start
Basic
Reduction
Processing
Export
Results
New Session_7
450nm
curve fit
Parameters
Graph
Table
List
Show options
Export
Open in Excel
Print
Multiskan GO SIMULATOR - Connected
Disconnect
Target: 20.0°C
Plate: 20.4°C
Cuvette: 21.1°C
admin

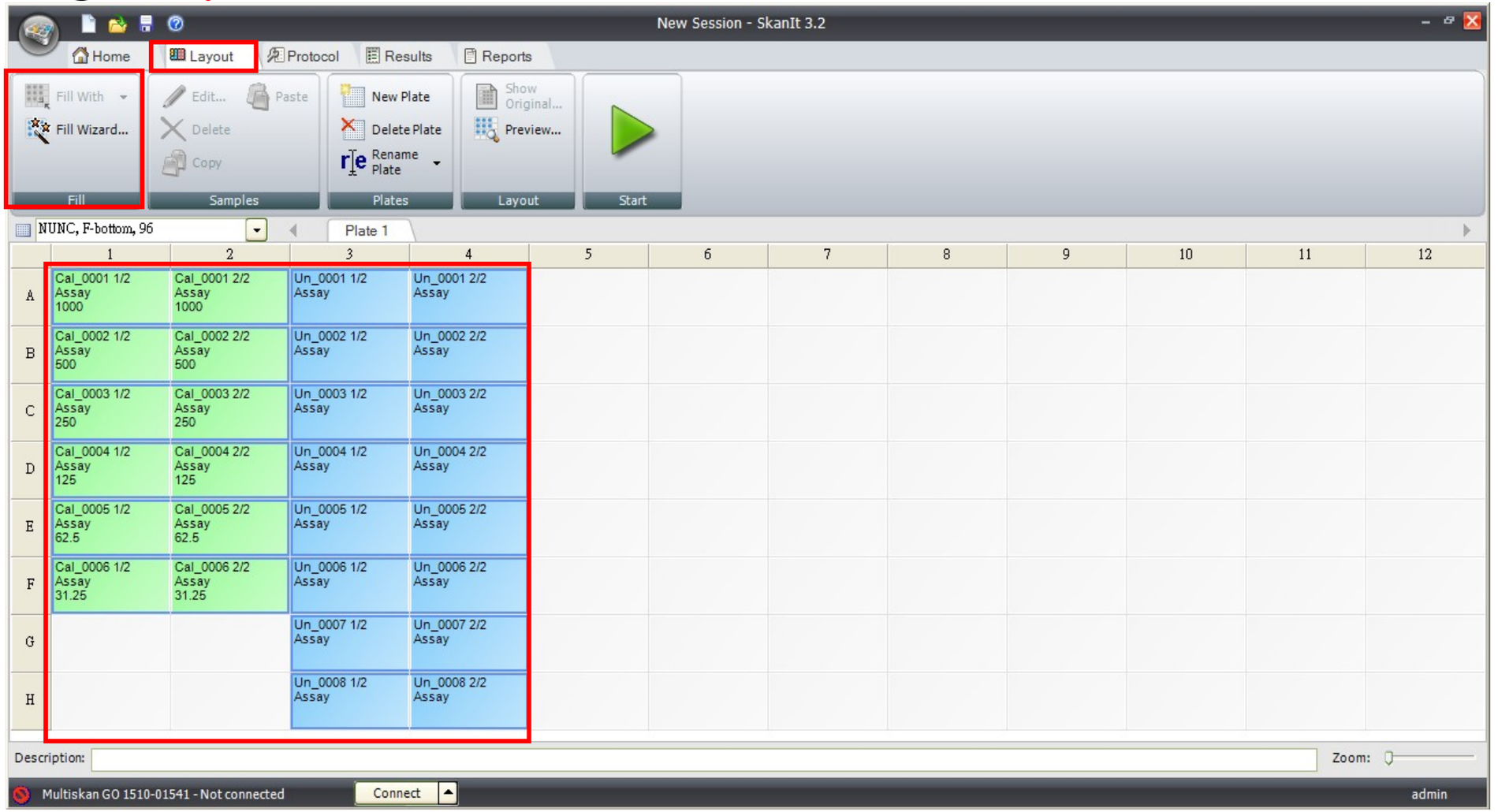
Operation-Plate measurement

- **Setting “Microplate”**



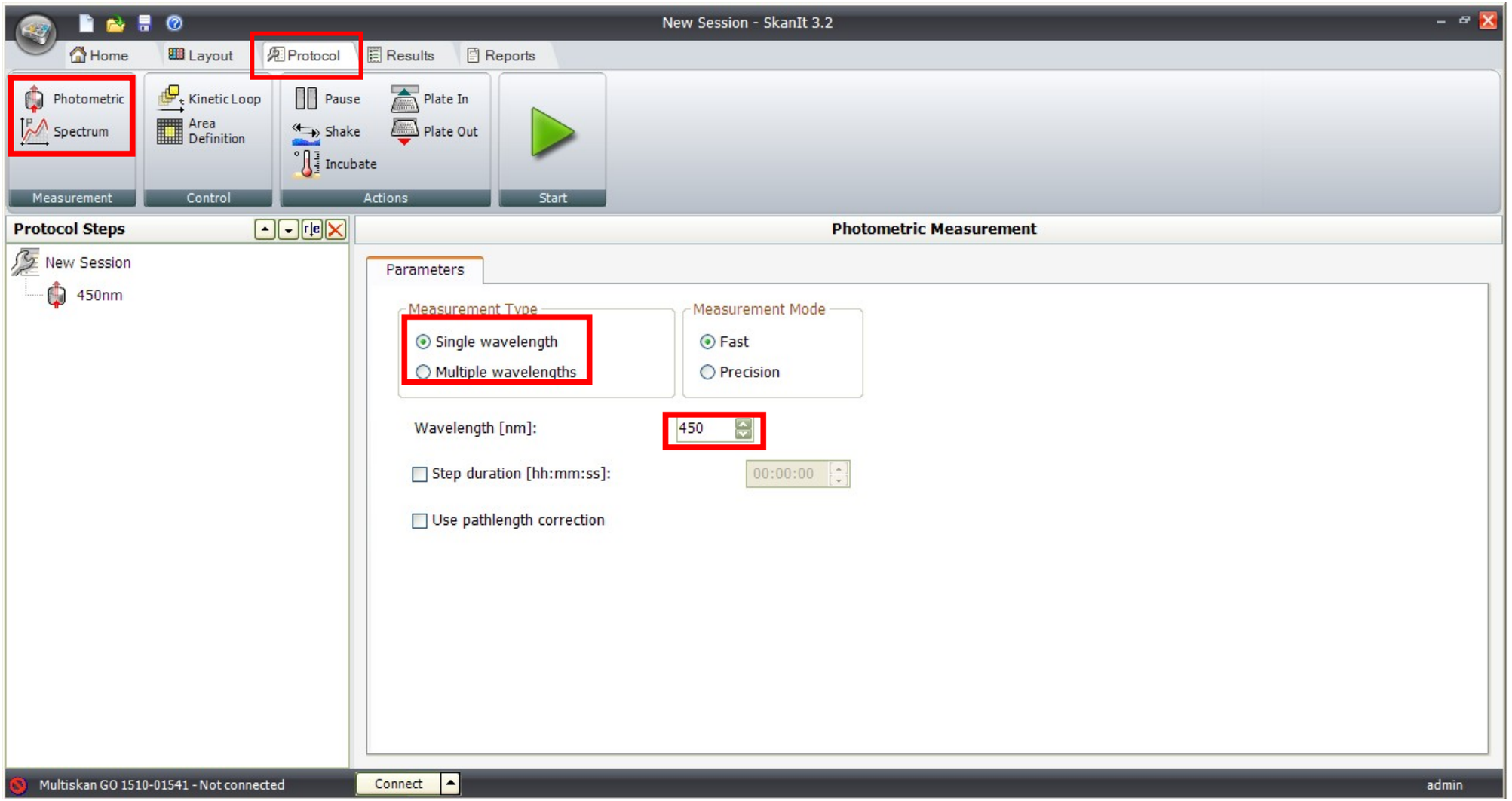
Operation-Plate measurement

- **Setting "Layout"**



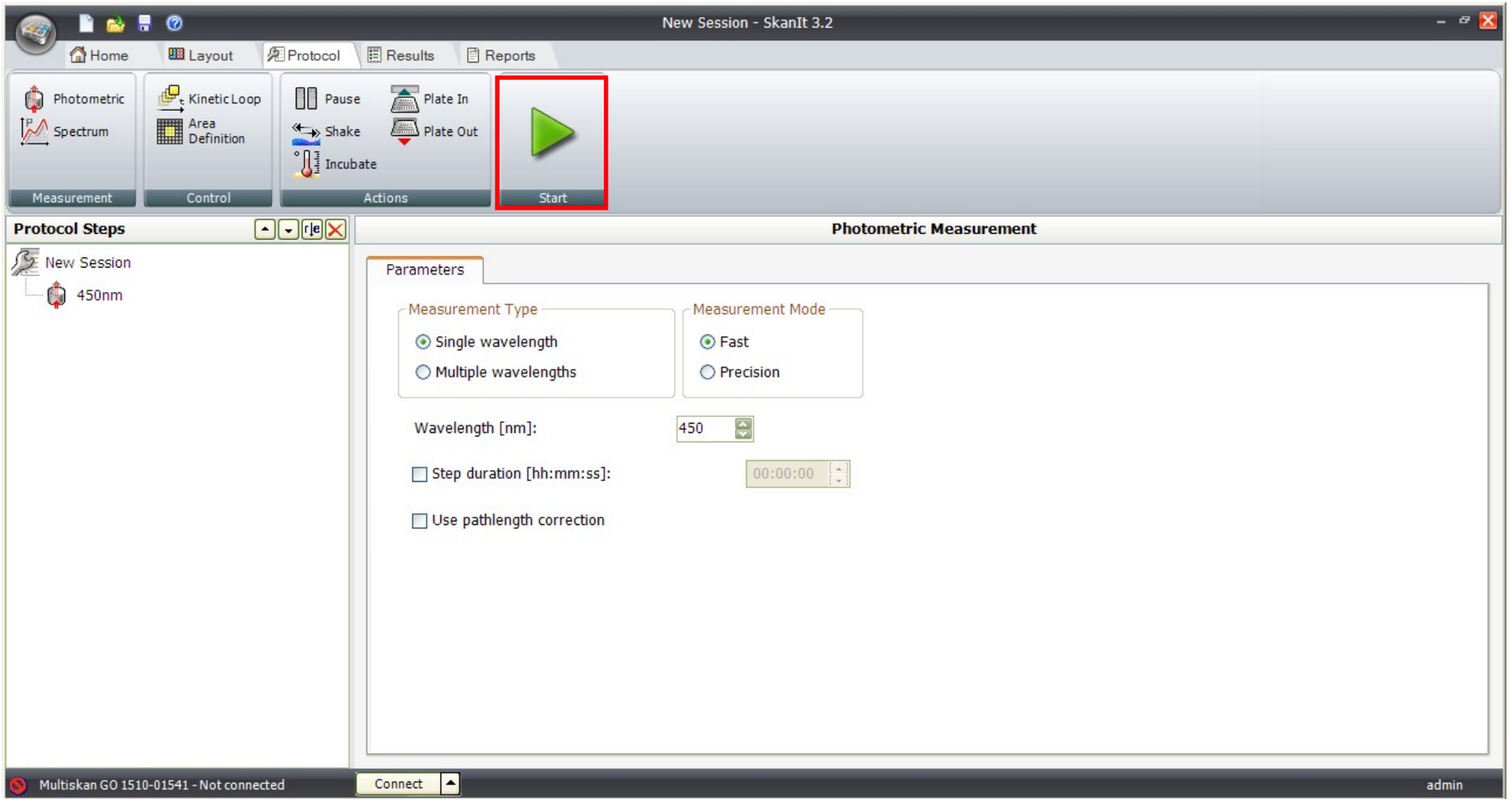
Operation-Plate measurement

- **Setting "Wavelength"**



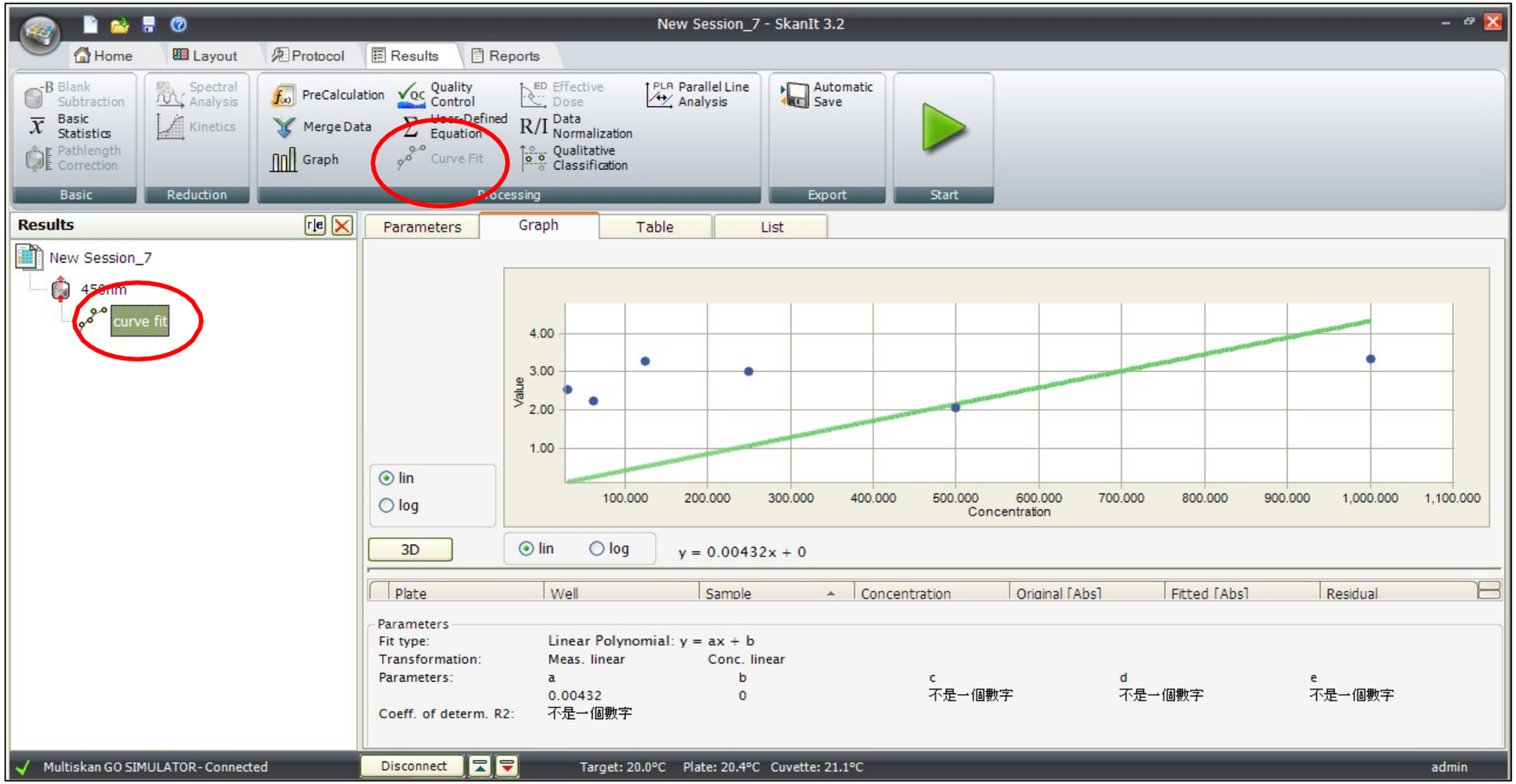
Operation-Plate measurement

- **“START“ Measurement**



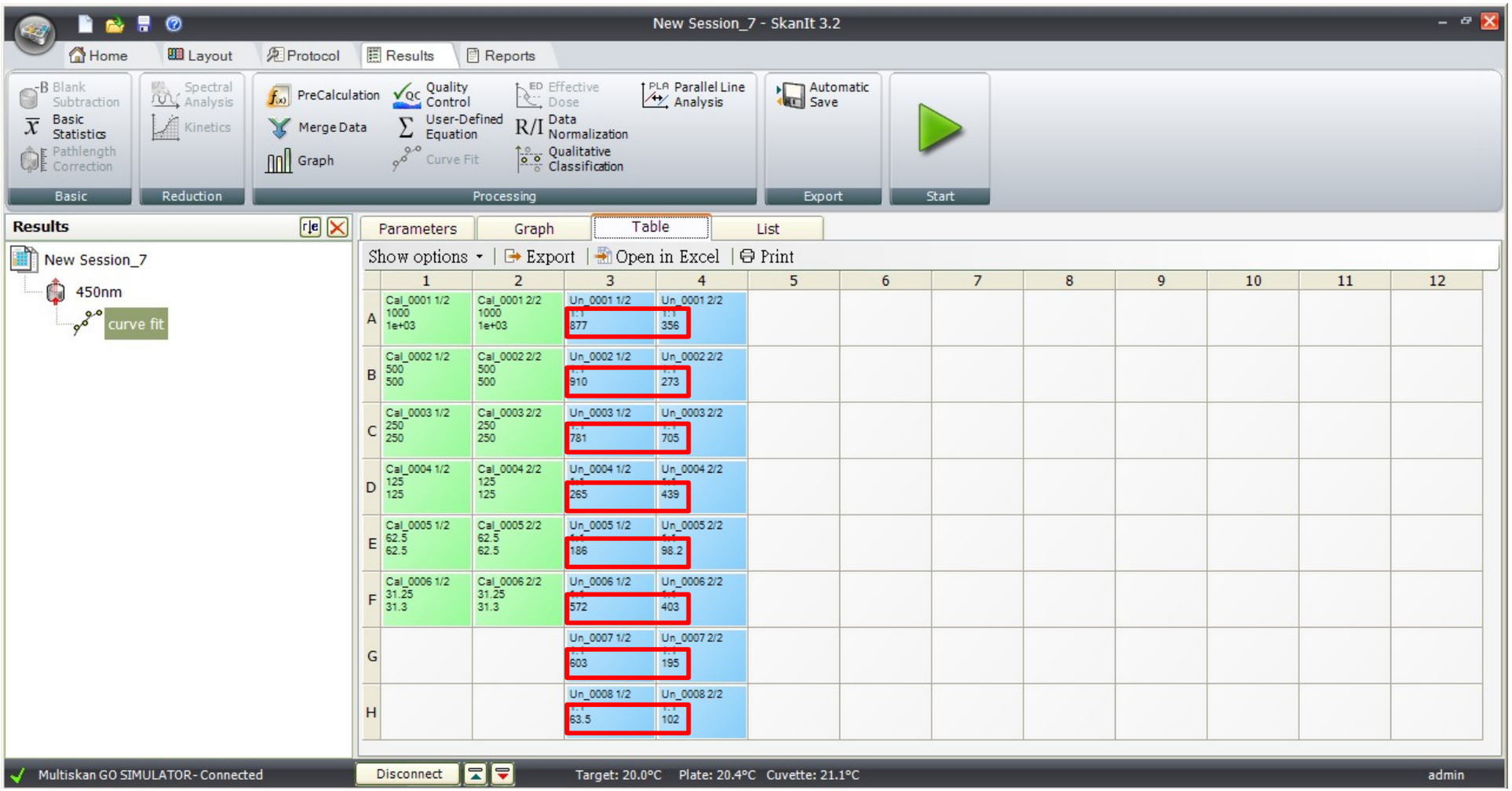
Operation

- Using “Curve fit” icon



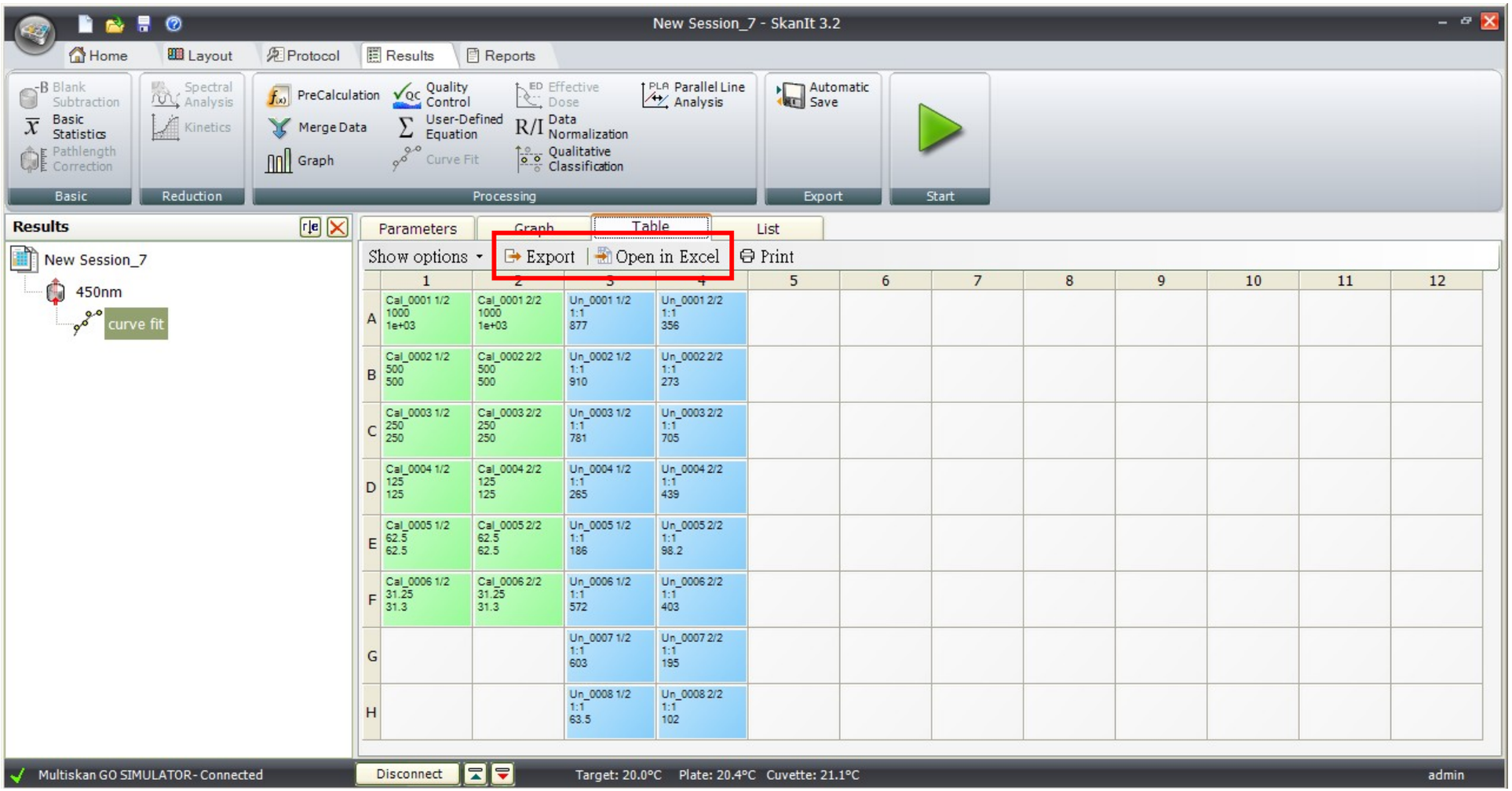
Operation-Plate measurement

- Sample concentration will be calculate **automatically**



Operation-Plate measurement

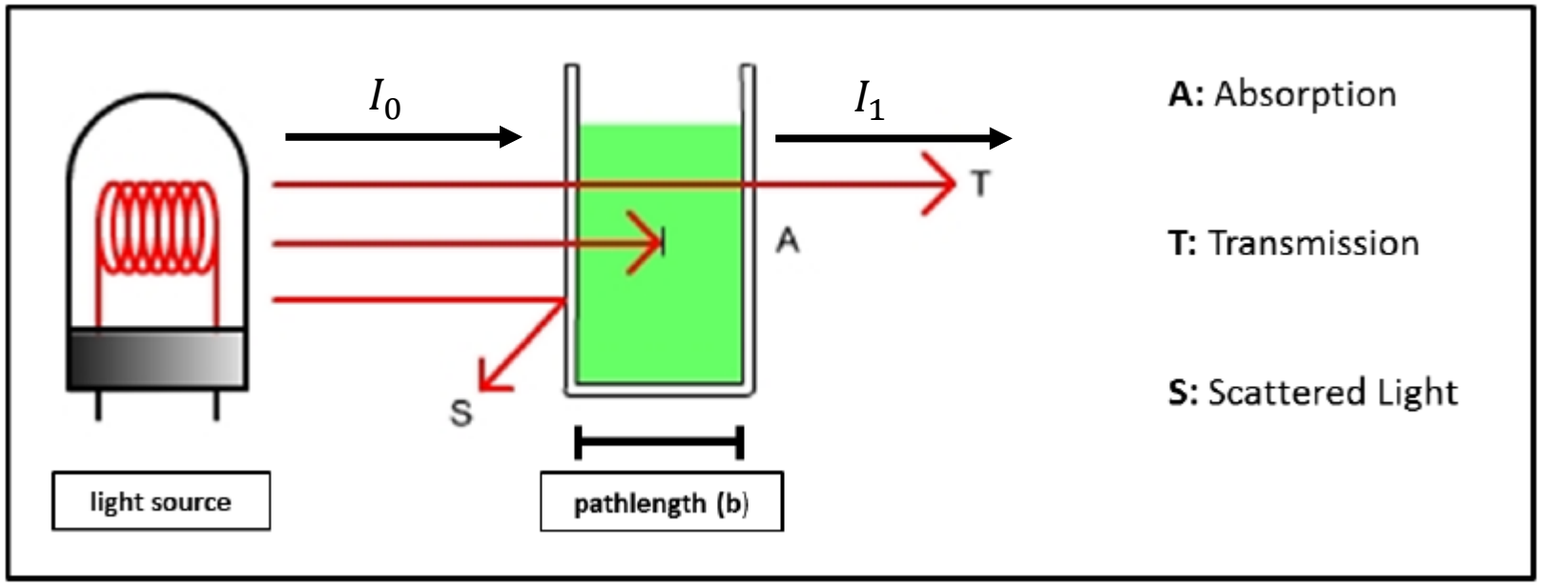
- **“Export“ Result**



岑 祥
TSENG HSIANG

Summary

Absorbance



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c	Molar concentration	M
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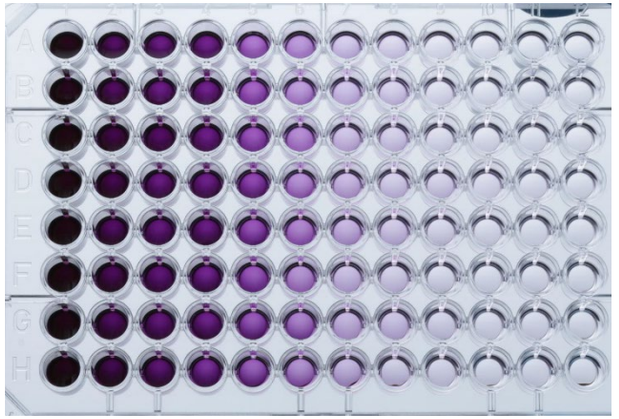
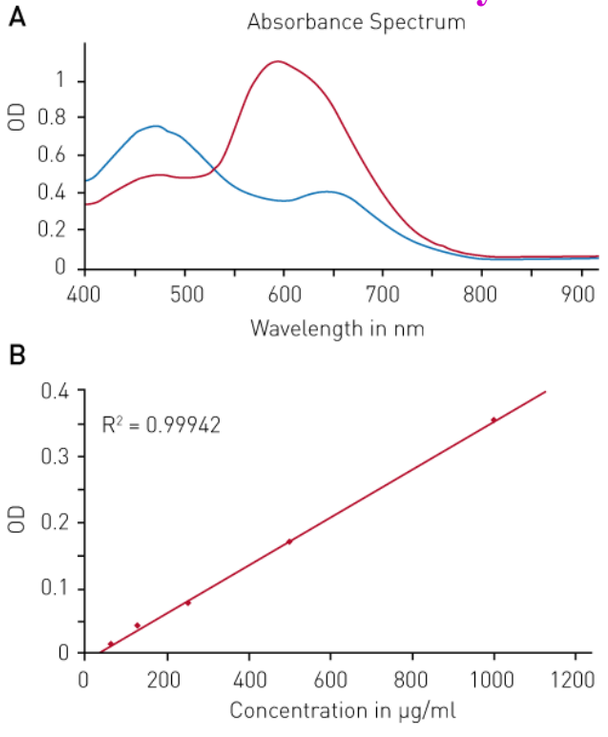
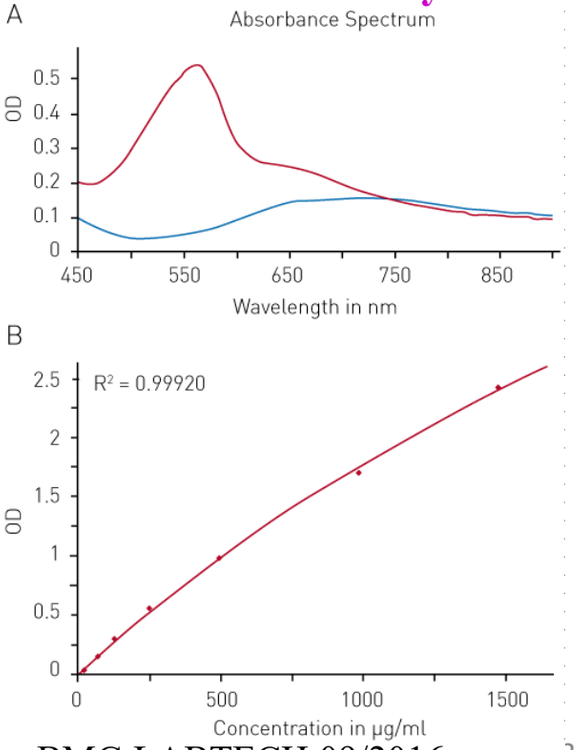


Cell viability(MTT Assay)

Bradford assay



BCA assay



~Thank You for Your Attention~