



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

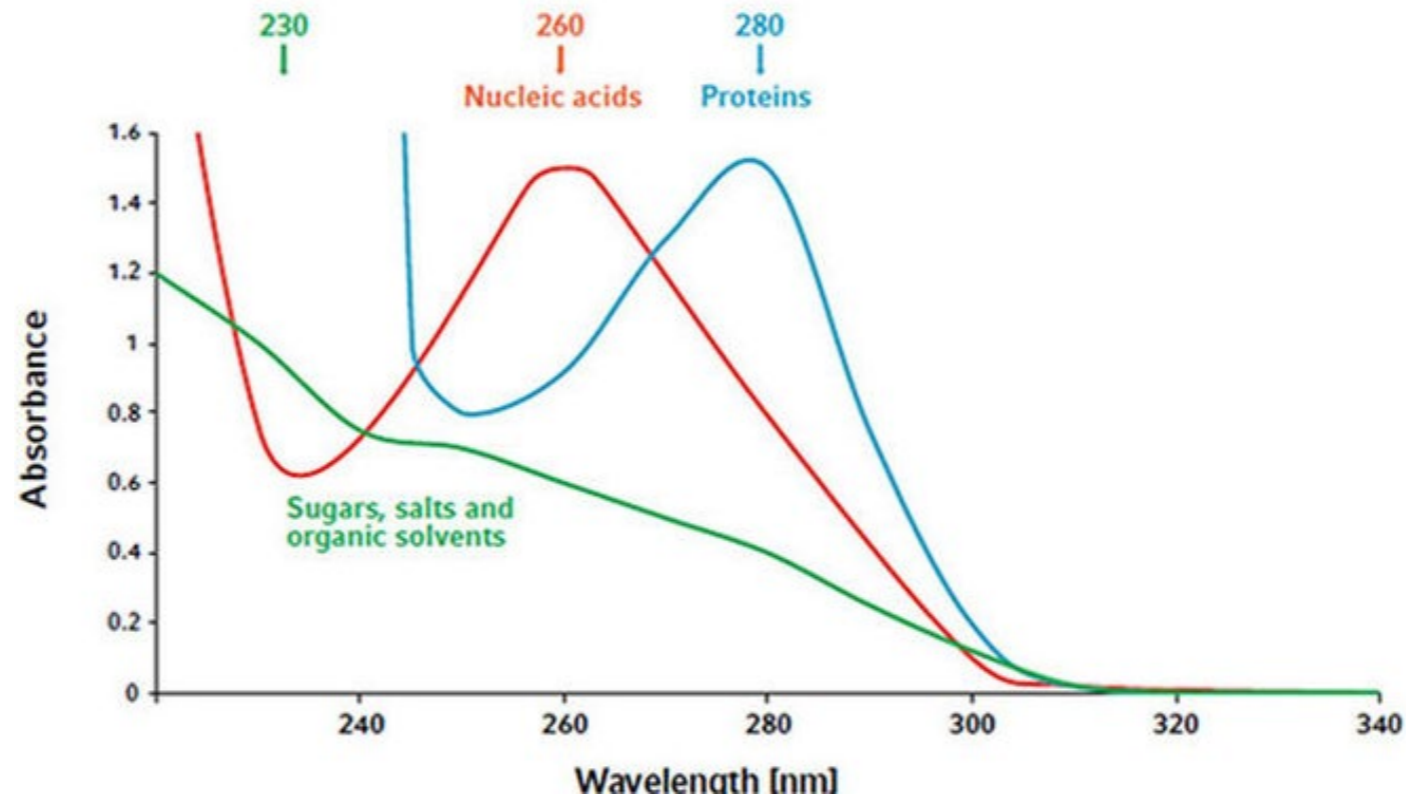


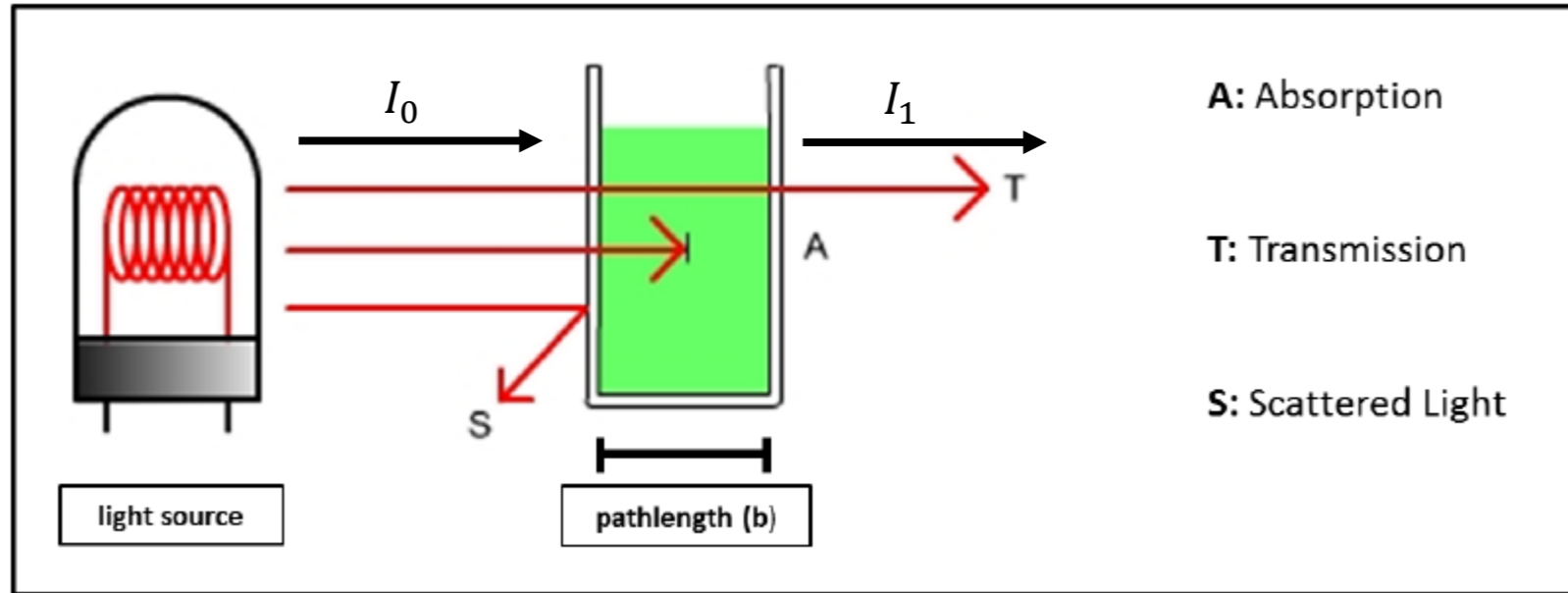
- Absorbance
- Application
- Introduction of Multiskan GO
- Operation



Why measure absorbance ?

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





$$\text{Transmittance}[T] = \frac{I_1}{I_0}$$

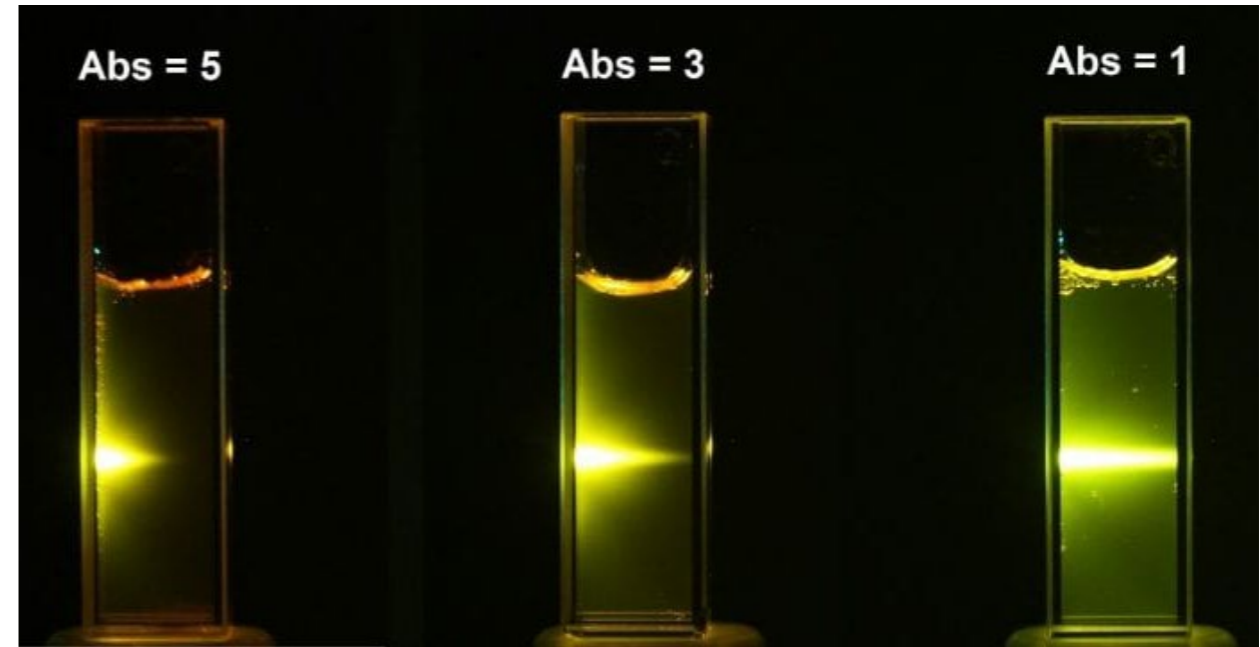
$$\text{Absorbance}[A] = -\log_{10} \left[\frac{I_0}{I_1} \right] = -\log_{10} \left[\frac{1}{T} \right]$$



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

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



<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

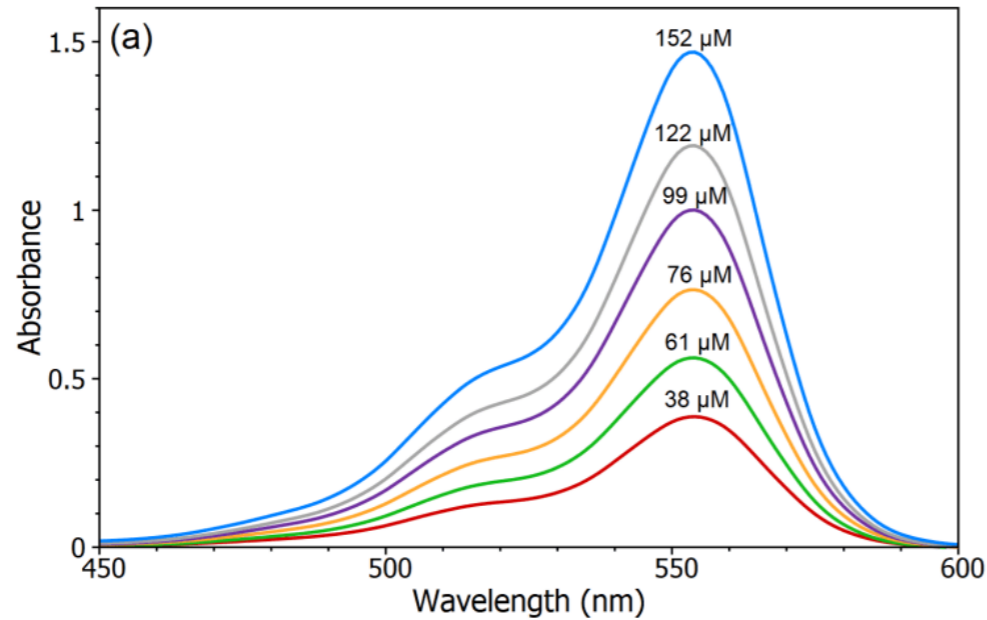


The Beer's law

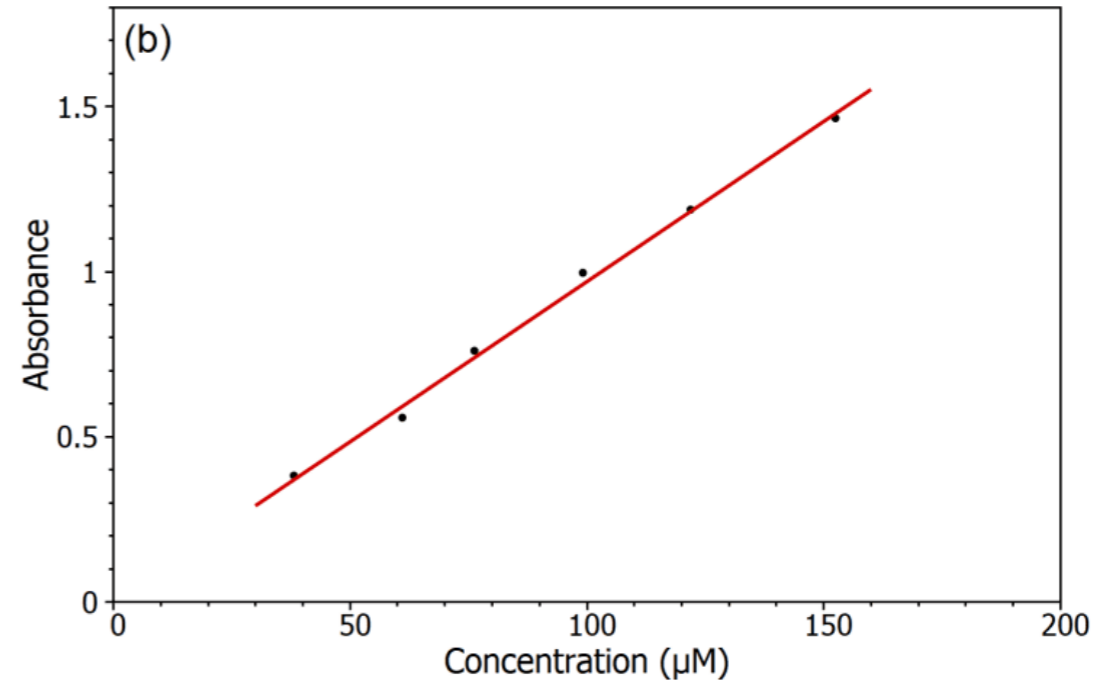
$$A = \epsilon cl$$

A
 ϵ
 c
 l

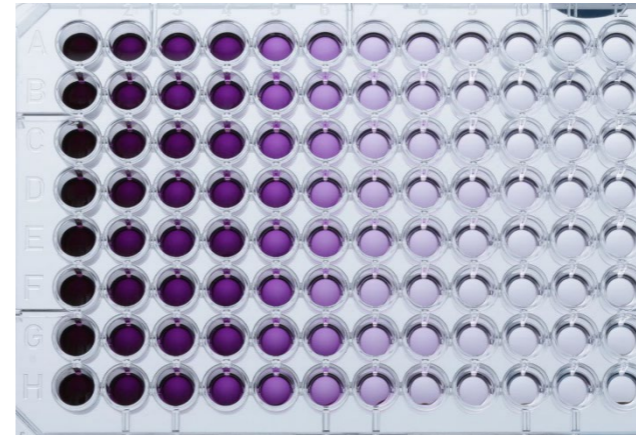
Absorbance	
Molar absorption coefficient	$M^{-1}cm^{-1}$
Molar concentration	M
optical path length	cm



Standard curve

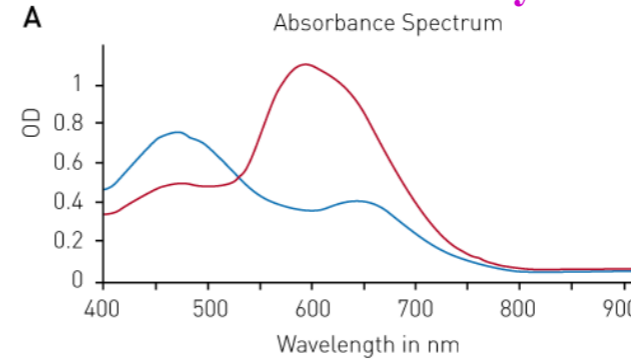


- ELISA
- Cell viability(MTT)
- BCA/Bradford
- DNA/Protein (260 nm / 280 nm)
- Microbial growth (OD 600 nm)

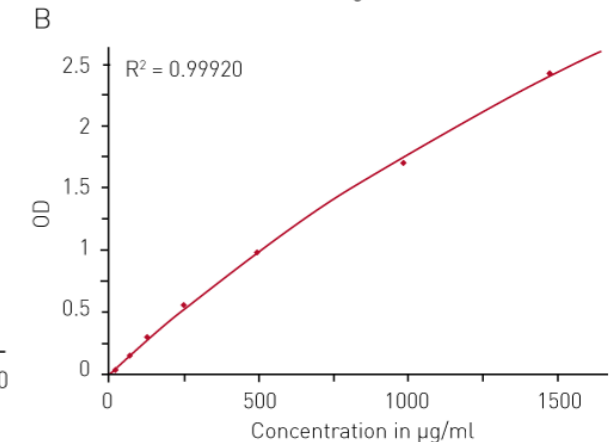
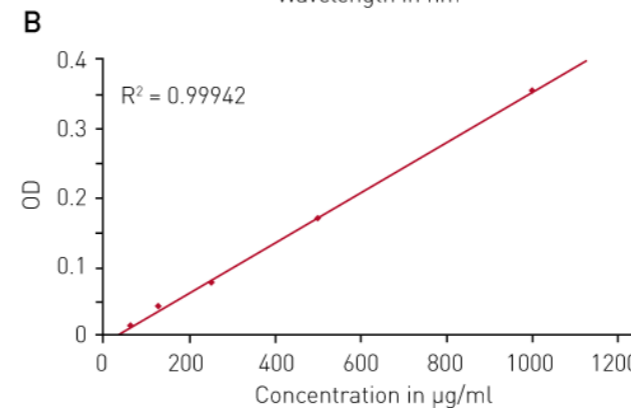
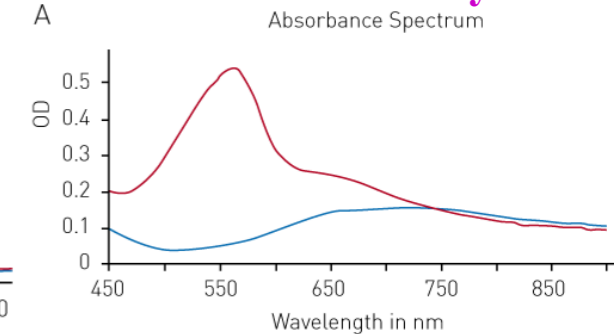


Cell viability(MTT Assay)

Bradford assay



BCA assay

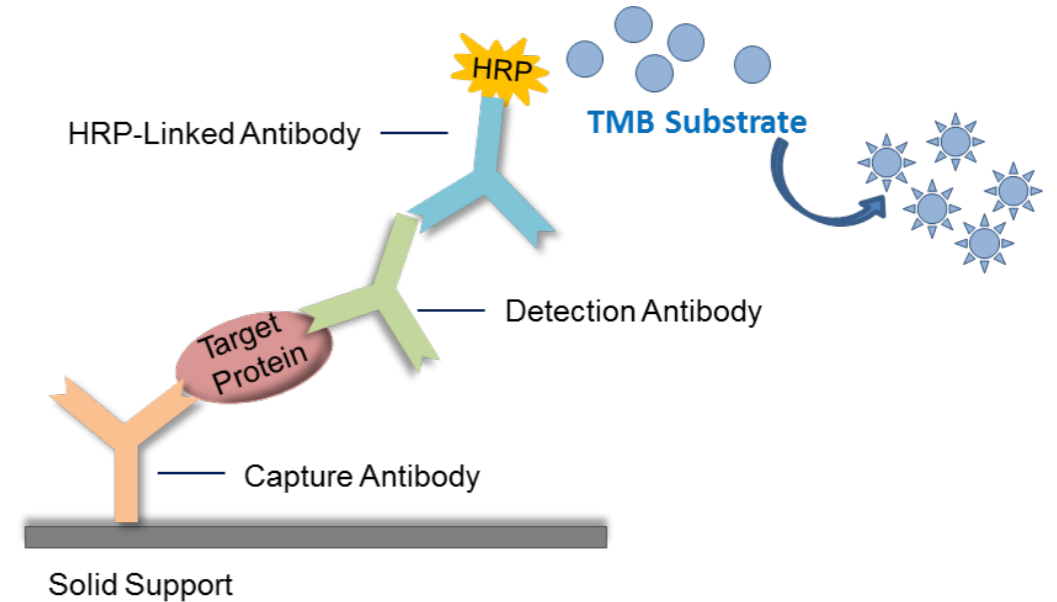




ELISA

To detect specific protein by antibody

Usually measure 450 or 405 nm



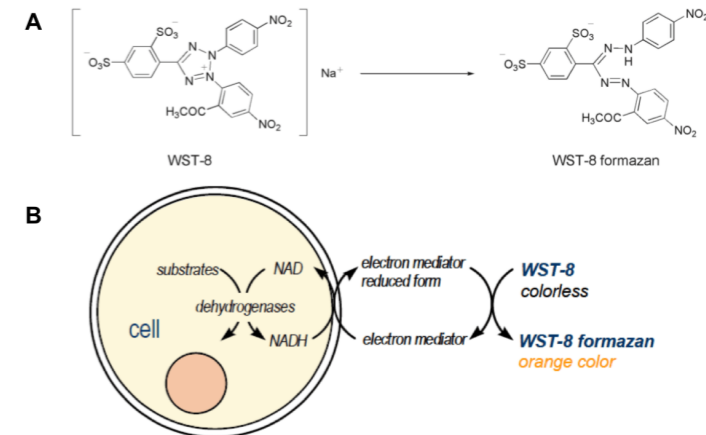
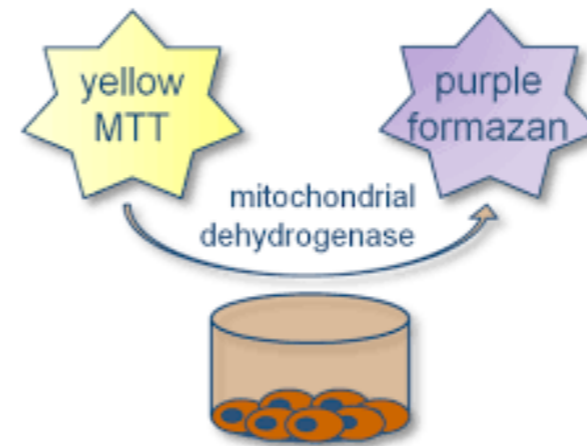
Cell viability CCK-8 AND MTT

To check the vial cell number by specific reagent

Usually measure 450 and 570 nm

MTT = 570nm

CCK-8 = 450nm

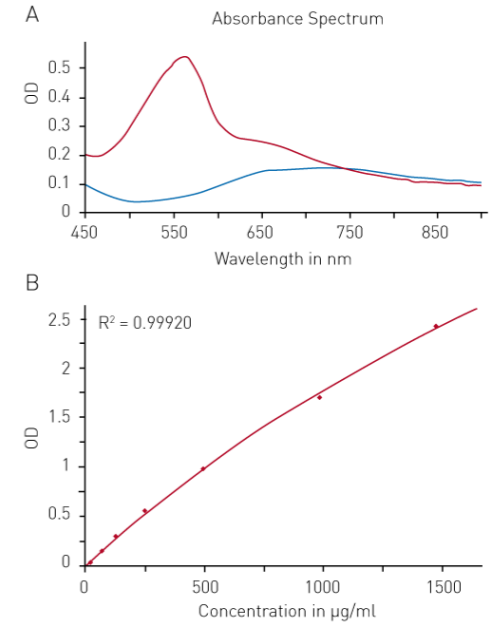
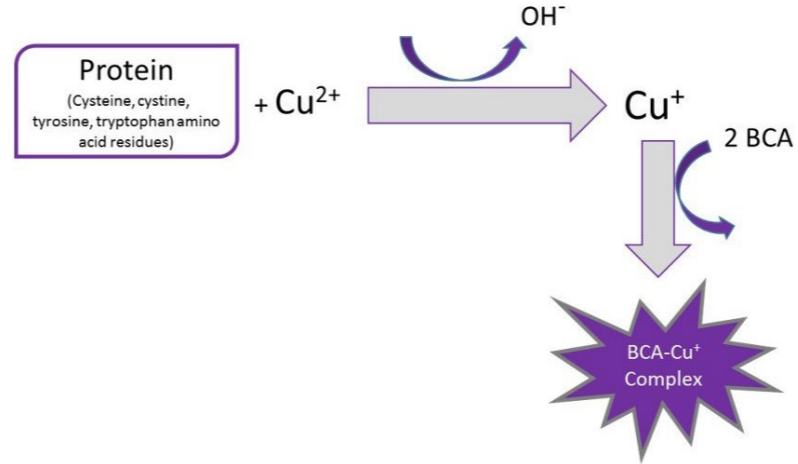




BCA

To quantify protein in unknown sample

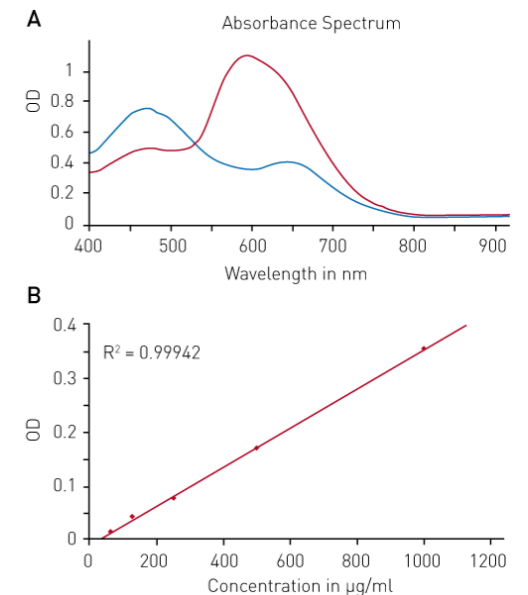
Usually measure 562 nm



Brafford

To quantify protein in unknown sample

Usually measure 595 nm





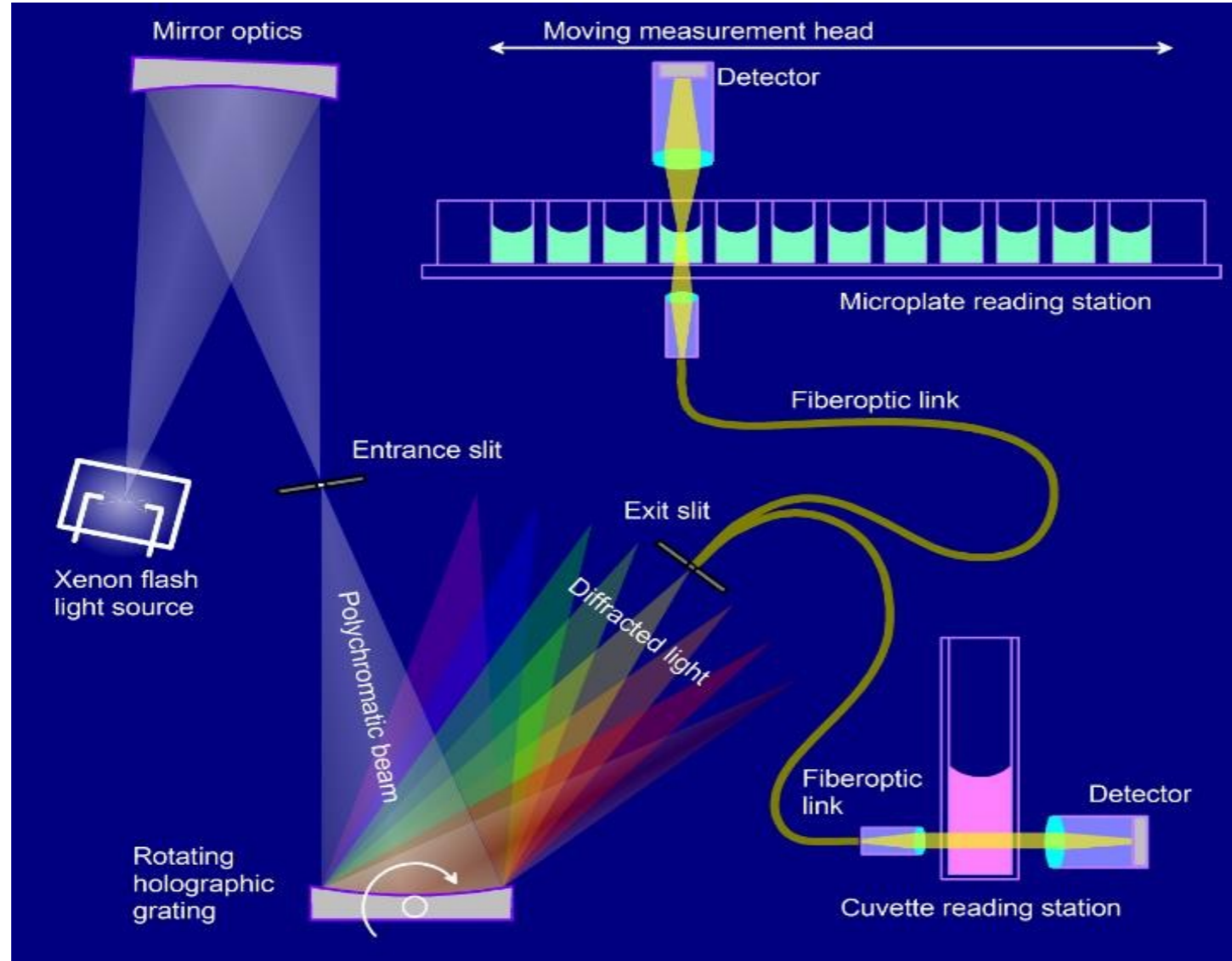
Multiskan GO

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



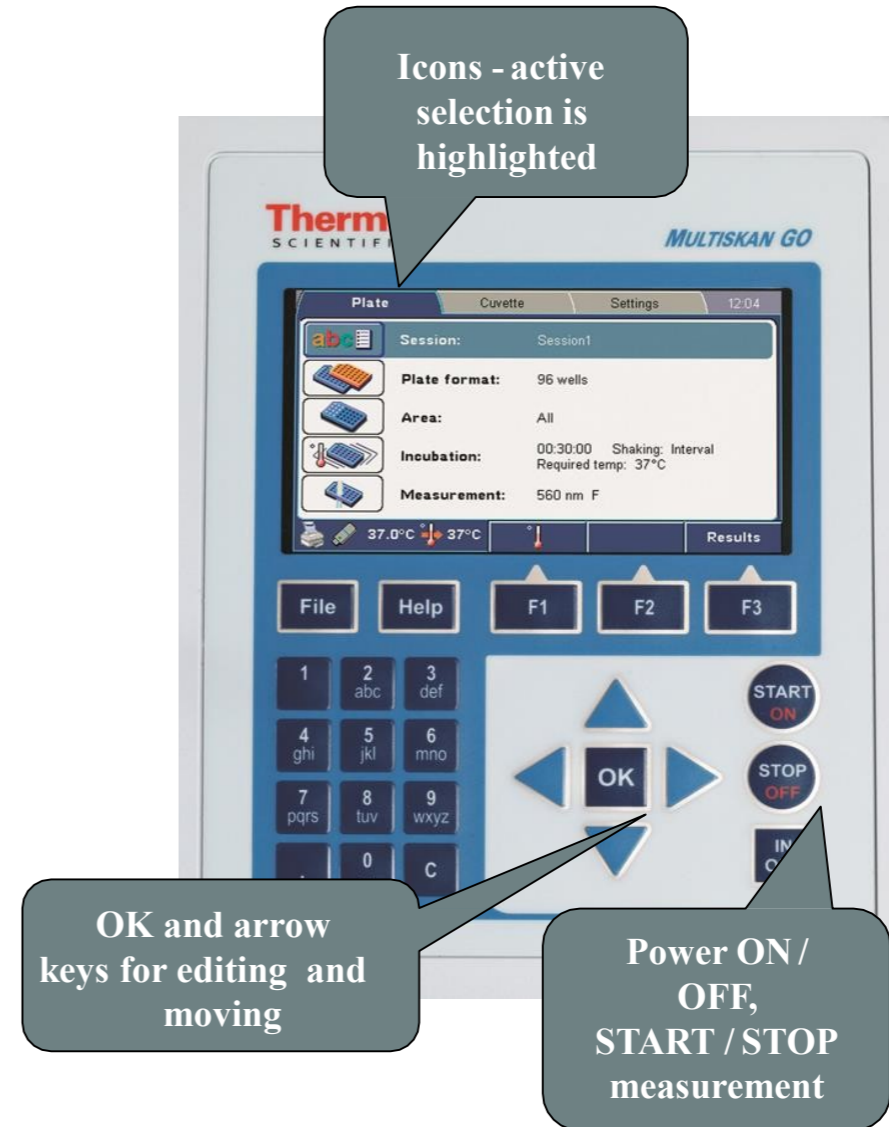


Monochromator based system with adjustable wavelength





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



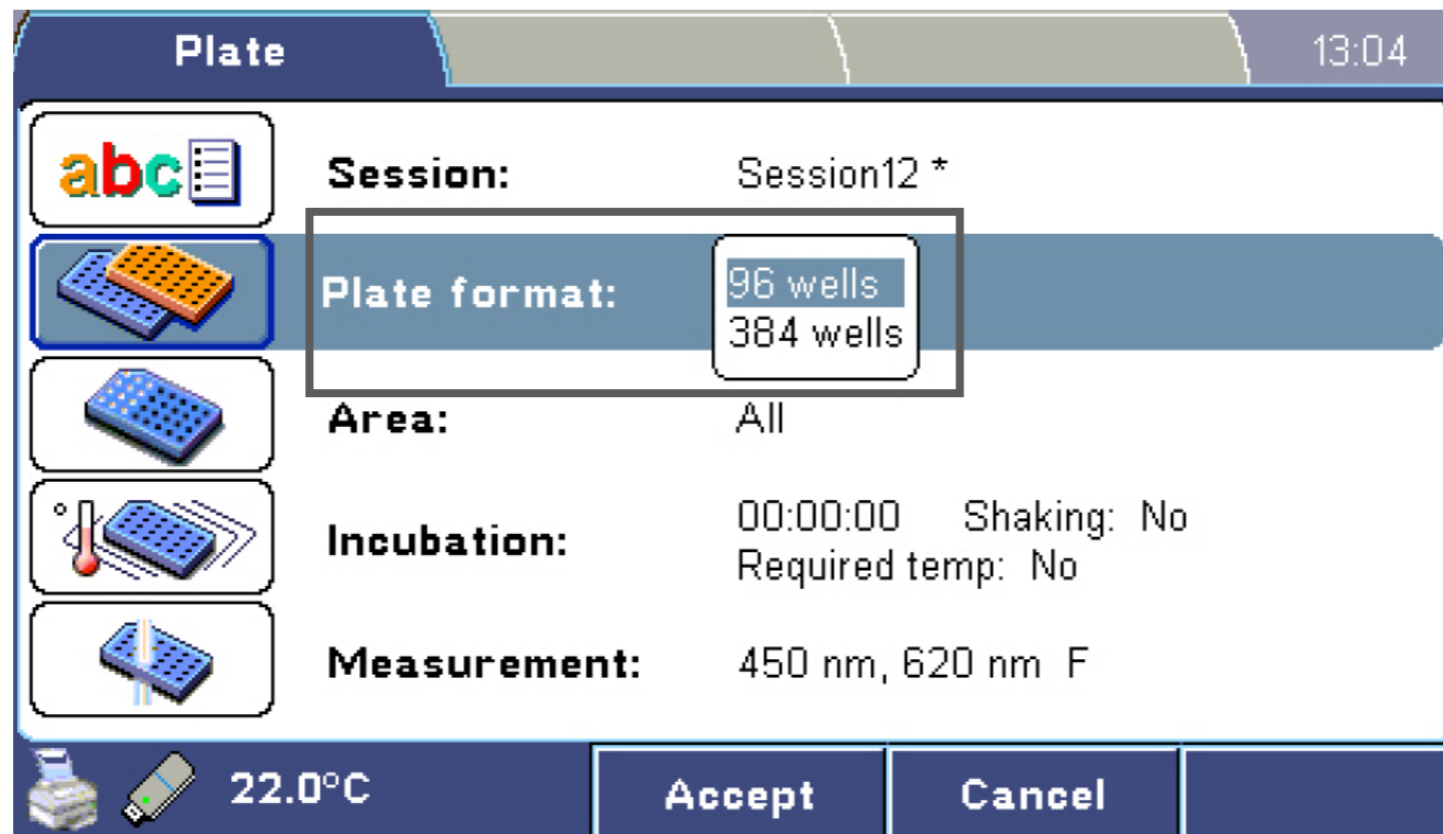


The image shows the control panel of a Thermo Scientific MULTISKAN GO plate reader. The screen displays a menu with tabs for 'Plate', 'Cuvette', and 'Settings', along with a clock showing '12.04'. The 'Plate' tab is active, showing session information: 'Session: Session1', 'Plate format: 96 wells', 'Area: All', 'Incubation: 00:30:00 Shaking: Interval Required temp: 37°C', and 'Measurement: 560 nm F'. Below the screen is a keypad with 'File', 'Help', 'F1', 'F2', and 'F3' function keys, a numeric keypad, a central 'OK' button with directional arrows, and 'START ON', 'STOP OFF', and 'IN OUT' buttons. Callouts provide details on these elements:

- Info text bar:** Points to the top status bar of the screen.
- Help offers information on the current view:** Points to the 'Help' button on the keypad.
- File functions:** Points to the 'File' button on the keypad.
 - For Plate: New, Open, Save, Save as, Export, Print, Delete
 - For Cuvette: Export (new file), Export (append), Print
- Time:** Points to the clock display in the top right corner.
- F1, F2 and F3 keys are used for different purposes depending on SW level:** Points to the function keys on the keypad.



▪ Selecting **“Plate format”**





▪ Selecting “Area”

The screenshot shows the 'Plate' settings menu with the following options:

- Session: Session1
- Plate format: 96 wells
- Area: All
- Incubation: 00:00:00 Shaking: No Required temp: 37°C
- Measurement: 450 nm F

At the bottom, there is a temperature display showing 22.0°C and function keys for File, Help, F1, and F2.

The screenshot shows the 'Plate' selection screen with a 12x8 grid of wells. The selected area is highlighted in blue, corresponding to the range C3 - F7. The temperature display shows 22.0°C. At the bottom, there are buttons for 'Accept', 'Cancel', and 'Clear'.

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	■	■	■	■	■	○	○	○	○	○
D	○	○	■	■	■	■	○	○	○	○	○	○
E	○	○	■	■	■	■	○	○	○	○	○	○
F	○	○	■	■	■	■	■	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○









Selected area:
C3 - F7



Setting "Measurement"

Plate 13:04

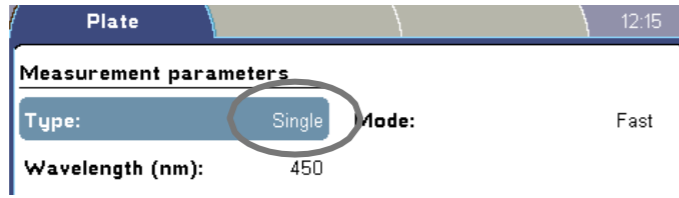
	Session: Session12 *
	Plate format: 96 wells 384 wells
	Area: All
	Incubation: 00:00:00 Shaking: No Required temp: No
	Measurement: 450 nm, 620 nm F

 22.0°C Accept Cancel

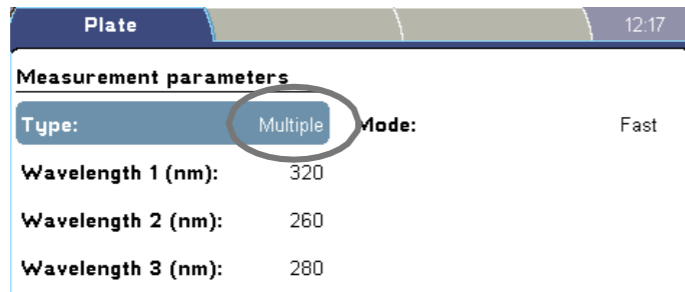


Measurement types

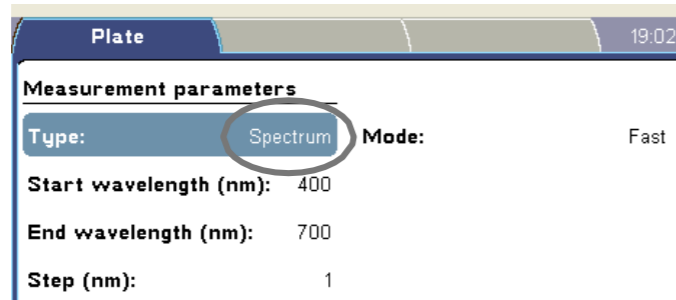
Single



Multiple



Spectrum



Use the arrow keys to scroll the view

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.014	0.112	0.276	0.089	0.295	0.316	0.126	-0.051	0.092	0.429	0.031	-0.018
B	0.167	0.022	0.082	0.629	-0.037	-0.018	1.867	0.099	-0.053	1.278	0.415	0.422
C	1.008	0.051	0.318	0.625	0.273	0.567	0.188	0.563	-0.043	0.050	1.020	0.841
D	0.370	-0.007	0.272	-0.007	0.214	0.507	0.780	0.157	1.099	0.056	0.344	0.438
E	0.175	0.076	0.559	0.416	0.360	0.321	0.077	0.911	0.105	0.346	0.024	0.234
F	-0.027	0.425	0.757	0.968	0.481	0.347	1.533	-0.003	0.453	0.015	0.744	0.012
G	0.247	0.133	0.591	0.324	0.163	0.109	0.860	0.289	0.799	0.034	0.111	0.226
H	0.418	0.241	0.224	1.021	0.075	1.488	0.200	0.422	0.135	0.017	0.642	

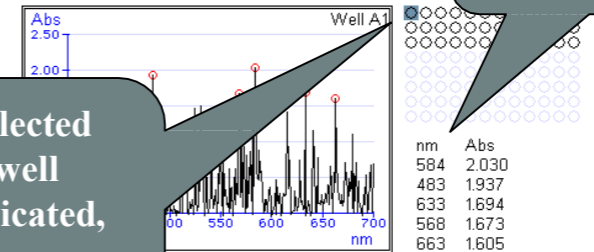
Result for each wavelength

320 nm, 260 nm, 280 nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.329	0.310	0.474	1.105	0.040	0.241	-0.001	1.080	0.079	1.883	0.880	0.409
B	0.520	0.335	0.432	0.840	0.050	0.537	1.311	-0.040	0.439	0.428	0.662	0.465
C	0.058	0.187	0.126	0.701	1.037	0.531	-0.009	0.468	-0.021	0.134	1.536	0.094
D	0.314	0.044	0.012	0.211	0.179	0.148	0.072	-0.002	0.812	0.169	0.306	0.142
E	0.215	0.229	-0.045	-0.049	0.234	0.041	0.241	-0.003	0.065	0.250	0.303	0.449
F	0.217	0.409	0.506	0.446	0.155	0.062	0.504	0.395	0.225	0.039	0.260	0.276
G	0.380	0.992	0.061	0.423	-0.012	0.162	1.352	0.618	0.094	0.304	0.817	0.538
H	0.190	0.046	-0.017	0.414	0.163	1.436	0.598	0.381	-0.042	-0.001	0.035	0.008
I	0.210	-0.005	0.054	0.194	0.161	0.139	0.253	0.523	1.876			
J	1.072	0.333	0.567	0.732	2.052	1.474	0.228	0.328	-0.03			
K	0.168	0.555	0.233	0.208	0.571	0.022	0.440	0.760	0.09			
L	0.798	0.488	-0.048	0.294	-0.049	0.794	0.150	0.730	0.60			

Up to five largest peak values

Selected well indicated, use arrow keys to move





- **“START”** Measurement





▪ “Saving” Result

Plate 11:44

test4 450 nm F

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.050	0.679	0.148	0.543	0.774	0.265	0.671	0.379	0.215	0.055	0.584	0.522
B	0.126	0.112	1.100	0.056	0.722	0.161	0.166	0.112	0.188	0.85		
C	0.218	1.297	0.114	0.197	0.183	0.043	0.490	0.243	0.442	0.19		
D	0.167	0.090	0.046	0.199	0.071	1.081	0.306	0.346	0.240	0.40		
E	0.643	0.313	0.284	0.423	0.145	0.880	0.259	0.751	0.853	0.19		
F	0.091	0.291	0.094	0.235	0.445	0.740	0.543	0.127	0.288	0.640		
G	0.337	0.634	0.375	0.046	0.054	0.769	0.772	0.058	0.441	0.475		0.401
H	0.163	0.161	0.270	1.391	0.130	0.640	1.542	0.492	0.15	0.548	1.007	0.290

22.1°C

File Help Export Save As Close

F1 F2 F3

USB flash memory device connected

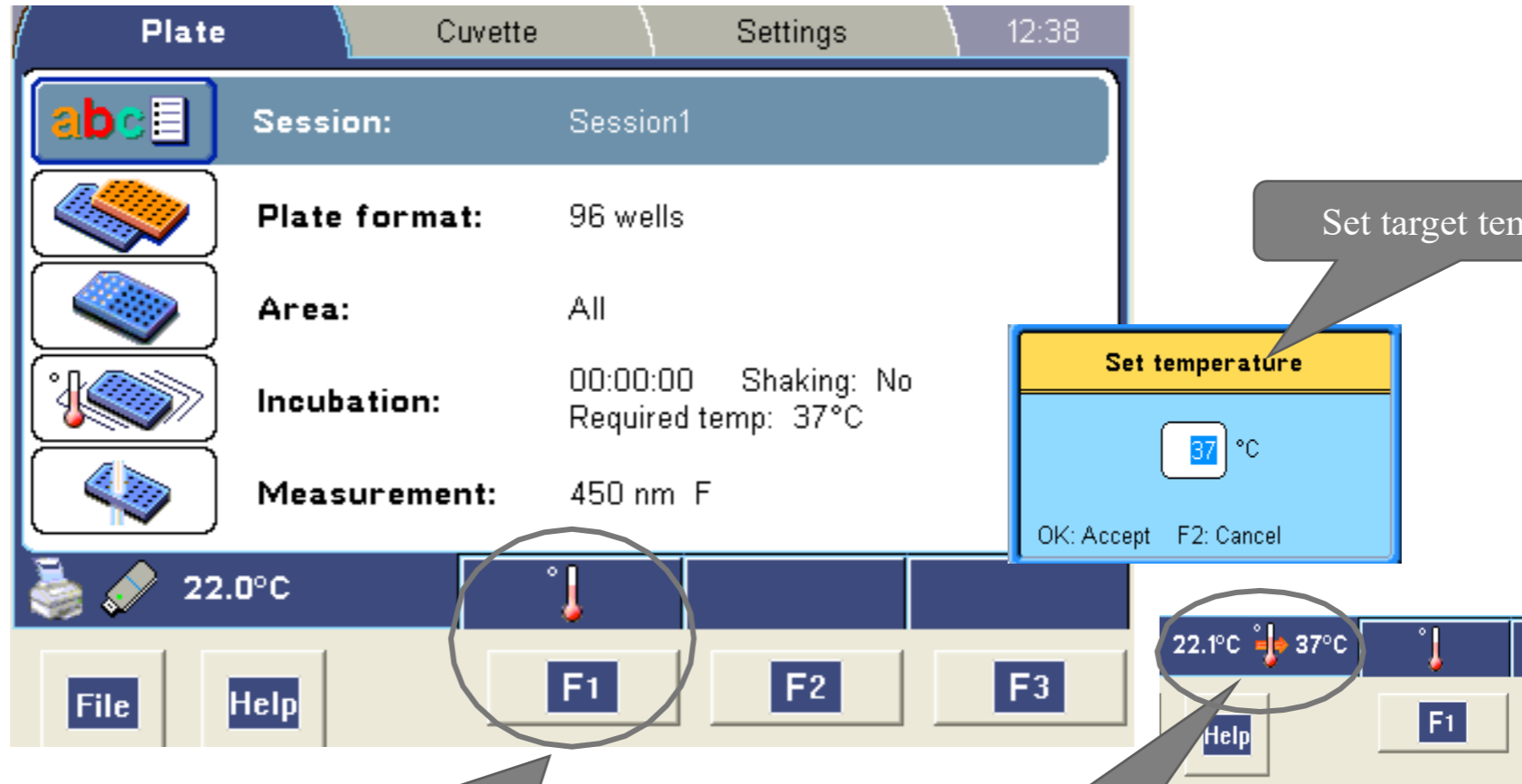
- New
- Open
- Save
- Save As
- Export
- Print
- Delete

- Export plate Session(s)
 - Export current session
 - F1 (Export)

- Save the plate Session:
 - F2 (Save as)
 - File -> Save or Save as



■ “Incubation”



■ The incubator temperature can be controlled using F1 key

Instrument and Target temperature

Set target temperature

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



New Session - SkanIt 3.2

Home | Layout | Protocol | Results | Reports

Fill With | Edit... | Paste | New Plate | Show Original... | Start

Fill Wizard... | Delete | Copy | Delete Plate | Rename Plate | Preview...

Fill | Samples | Plates | Layout | Start

NUNC, F-bottom, 96 | Plate 1

	1	2	3	4	5	6	7	8	9
A	CaL_0001 1/2 Assay 1000	CaL_0001 2/2 Assay 1000	Un_0001 1/2 Assay	Un_0001 2/2 Assay					
B	CaL_0002 1/2 Assay 500	CaL_0002 2/2 Assay 500	Un_0002 1/2 Assay	Un_0002 2/2 Assay					
C	CaL_0003 1/2 Assay 250	CaL_0003 2/2 Assay 250	Un_0003 1/2 Assay	Un_0003 2/2 Assay					
D	CaL_0004 1/2 Assay 125	CaL_0004 2/2 Assay 125	Un_0004 1/2 Assay	Un_0004 2/2 Assay					
E	CaL_0005 1/2 Assay 62.5	CaL_0005 2/2 Assay 62.5	Un_0005 1/2 Assay	Un_0005 2/2 Assay					
F	CaL_0006 1/2 Assay 31.25	CaL_0006 2/2 Assay 31.25	Un_0006 1/2 Assay	Un_0006 2/2 Assay					
G			Un_0007 1/2 Assay	Un_0007 2/2 Assay					
H			Un_0008 1/2 Assay	Un_0008 2/2 Assay					

Description:

Multiskan GO 1510-01541 - Not connected | Connect

New Session_7 - SkanIt 3.2

Home | Layout | Protocol | Results | Reports

Blank Subtraction | Spectral Analysis | PreCalculation | QC | Effective Dose | PLA Parallel Line Analysis | Automatic Save

Basic Statistics | Kinetics | Merge Data | User-Defined Equation | R/I Data Normalization

Pathlength Correction | Graph | Curve Fit | Qualitative Classification

Basic | Reduction | Processing | Export | Start

Results | Parameters | Graph | Table | List

New Session_7

450nm curve fit

Show options | Export | Open in Excel | Print

	1	2	3	4	5	6	7	8	9	10	11	12
A	CaL_0001 1/2 1000 1e+03	CaL_0001 2/2 1000 1e+03	Un_0001 1/2 1.1 877	Un_0001 2/2 1.1 356								
B	CaL_0002 1/2 500 500	CaL_0002 2/2 500 500	Un_0002 1/2 1.1 910	Un_0002 2/2 1.1 273								
C	CaL_0003 1/2 250 250	CaL_0003 2/2 250 250	Un_0003 1/2 1.1 781	Un_0003 2/2 1.1 705								
D	CaL_0004 1/2 125 125	CaL_0004 2/2 125 125	Un_0004 1/2 1.1 265	Un_0004 2/2 1.1 439								
E	CaL_0005 1/2 62.5 62.5	CaL_0005 2/2 62.5 62.5	Un_0005 1/2 1.1 186	Un_0005 2/2 1.1 98.2								
F	CaL_0006 1/2 31.25 31.3	CaL_0006 2/2 31.25 31.3	Un_0006 1/2 1.1 572	Un_0006 2/2 1.1 403								
G			Un_0007 1/2 1.1 603	Un_0007 2/2 1.1 195								
H			Un_0008 1/2 1.1 63.5	Un_0008 2/2 1.1 102								

Multiskan GO SIMULATOR - Connected | Disconnect | Target: 20.0°C | Plate: 20.4°C | Cuvette: 21.1°C | admin



Setting “Microplate”

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: NUNC, F-bottom, 96

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal_0001 1/2 Assay 1000	Cal_0001 2/2 Assay 1000	Un_0001 1/2 Assay	Un_0001 2/2 Assay								
B	Cal_0002 1/2 Assay 500	Cal_0002 2/2 Assay 500	Un_0002 1/2 Assay	Un_0002 2/2 Assay								
C	Cal_0003 1/2 Assay 250	Cal_0003 2/2 Assay 250	Un_0003 1/2 Assay	Un_0003 2/2 Assay								
D	Cal_0004 1/2 Assay 125	Cal_0004 2/2 Assay 125	Un_0004 1/2 Assay	Un_0004 2/2 Assay								
E	Cal_0005 1/2 Assay 62.5	Cal_0005 2/2 Assay 62.5	Un_0005 1/2 Assay	Un_0005 2/2 Assay								
F	Cal_0006 1/2 Assay 31.25	Cal_0006 2/2 Assay 31.25	Un_0006 1/2 Assay	Un_0006 2/2 Assay								
G			Un_0007 1/2 Assay	Un_0007 2/2 Assay								
H			Un_0008 1/2 Assay	Un_0008 2/2 Assay								

Description: Zoom:

Multiskan GO 1510-01541 - Not connected admin



Setting "Layout"

The screenshot shows the SkanIt 3.2 software interface. The 'Layout' tab is selected in the top menu. The 'Fill Wizard...' button is highlighted with a red box. The main area displays a plate grid for 'Plate 1' with columns 1-12 and rows A-H. The grid contains assay names for wells A1-A8 and G3-H4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal_0001 1/2 Assay 1000	Cal_0001 2/2 Assay 1000	Un_0001 1/2 Assay	Un_0001 2/2 Assay								
B	Cal_0002 1/2 Assay 500	Cal_0002 2/2 Assay 500	Un_0002 1/2 Assay	Un_0002 2/2 Assay								
C	Cal_0003 1/2 Assay 250	Cal_0003 2/2 Assay 250	Un_0003 1/2 Assay	Un_0003 2/2 Assay								
D	Cal_0004 1/2 Assay 125	Cal_0004 2/2 Assay 125	Un_0004 1/2 Assay	Un_0004 2/2 Assay								
E	Cal_0005 1/2 Assay 62.5	Cal_0005 2/2 Assay 62.5	Un_0005 1/2 Assay	Un_0005 2/2 Assay								
F	Cal_0006 1/2 Assay 31.25	Cal_0006 2/2 Assay 31.25	Un_0006 1/2 Assay	Un_0006 2/2 Assay								
G			Un_0007 1/2 Assay	Un_0007 2/2 Assay								
H			Un_0008 1/2 Assay	Un_0008 2/2 Assay								

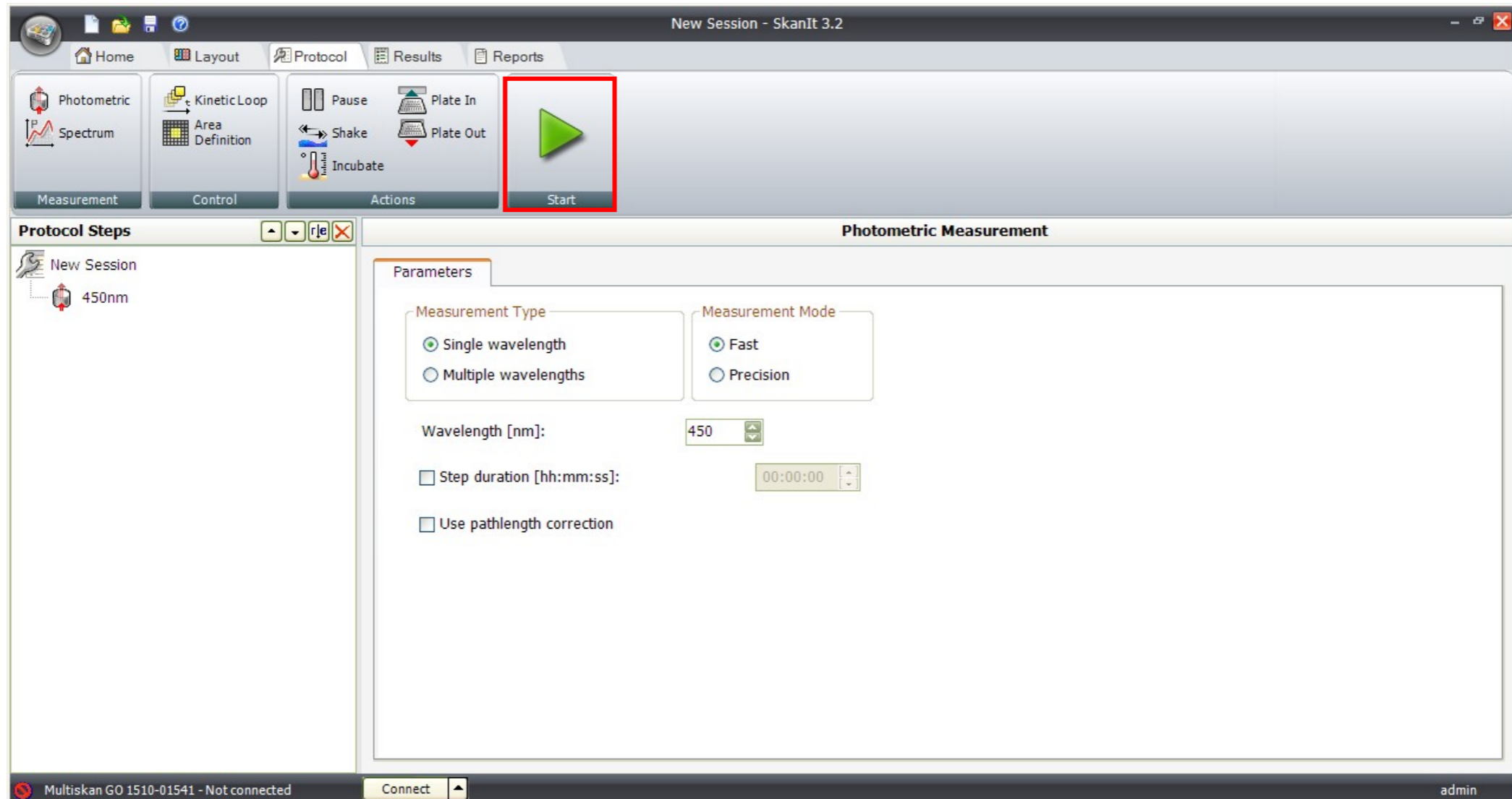


Setting “Wavelength”

The screenshot displays the SkanIt 3.2 software interface for a 'New Session'. The 'Protocol' tab is active, and the 'Photometric Measurement' parameters are shown. The 'Measurement Type' is set to 'Single wavelength', and the 'Wavelength [nm]' is set to 450. The 'Measurement Mode' is set to 'Fast'. The 'Step duration [hh:mm:ss]' is set to 00:00:00, and 'Use pathlength correction' is unchecked. The 'Protocol Steps' list shows a 'New Session' with a '450nm' measurement step. The status bar at the bottom indicates 'Multiskan GO 1510-01541 - Not connected' and the user is 'admin'.



■ “START” Measurement





- Using “Curve fit” icon

The screenshot shows the SkanIt 3.2 software interface. In the top toolbar, the 'Curve Fit' icon is circled in red. The 'Results' window is open, showing a graph of 'Value' vs 'Concentration' with a linear fit line. The fit equation is $y = 0.00432x + 0$. Below the graph, a table shows the fit parameters and their values.

Plate	Well	Sample	Concentration	Original [Abs]	Fitted [Abs]	Residual

Parameters

Fit type: Linear Polynomial: $y = ax + b$

Transformation: Meas. linear Conc. linear

Parameters:

a	b	c	d	e
0.00432	0	不是一個數字	不是一個數字	不是一個數字

Coeff. of determ. R2: 不是一個數字



- Sample concentration will be calculate **automatically**

The screenshot shows the SkanIt 3.2 software interface. The 'Results' window is open, displaying a table with 12 columns and 8 rows (A-H). The table is divided into columns 1-4 (Cal) and 5-12 (Un). The values in columns 3 and 4 are highlighted with red boxes, indicating they are calculated automatically.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal_0001 1/2 1000 1e+03	Cal_0001 2/2 1000 1e+03	Un_0001 1/2 111 877	Un_0001 2/2 111 356								
B	Cal_0002 1/2 500 500	Cal_0002 2/2 500 500	Un_0002 1/2 111 910	Un_0002 2/2 111 273								
C	Cal_0003 1/2 250 250	Cal_0003 2/2 250 250	Un_0003 1/2 111 781	Un_0003 2/2 111 705								
D	Cal_0004 1/2 125 125	Cal_0004 2/2 125 125	Un_0004 1/2 111 265	Un_0004 2/2 111 439								
E	Cal_0005 1/2 62.5 62.5	Cal_0005 2/2 62.5 62.5	Un_0005 1/2 111 186	Un_0005 2/2 111 98.2								
F	Cal_0006 1/2 31.25 31.3	Cal_0006 2/2 31.25 31.3	Un_0006 1/2 111 572	Un_0006 2/2 111 403								
G			Un_0007 1/2 111 603	Un_0007 2/2 111 195								
H			Un_0008 1/2 111 63.5	Un_0008 2/2 111 102								

At the bottom of the interface, the status bar shows: Multiskan GO SIMULATOR - Connected, Disconnect, Target: 20.0°C, Plate: 20.4°C, Cuvette: 21.1°C, and admin.



▪ “Export” Result

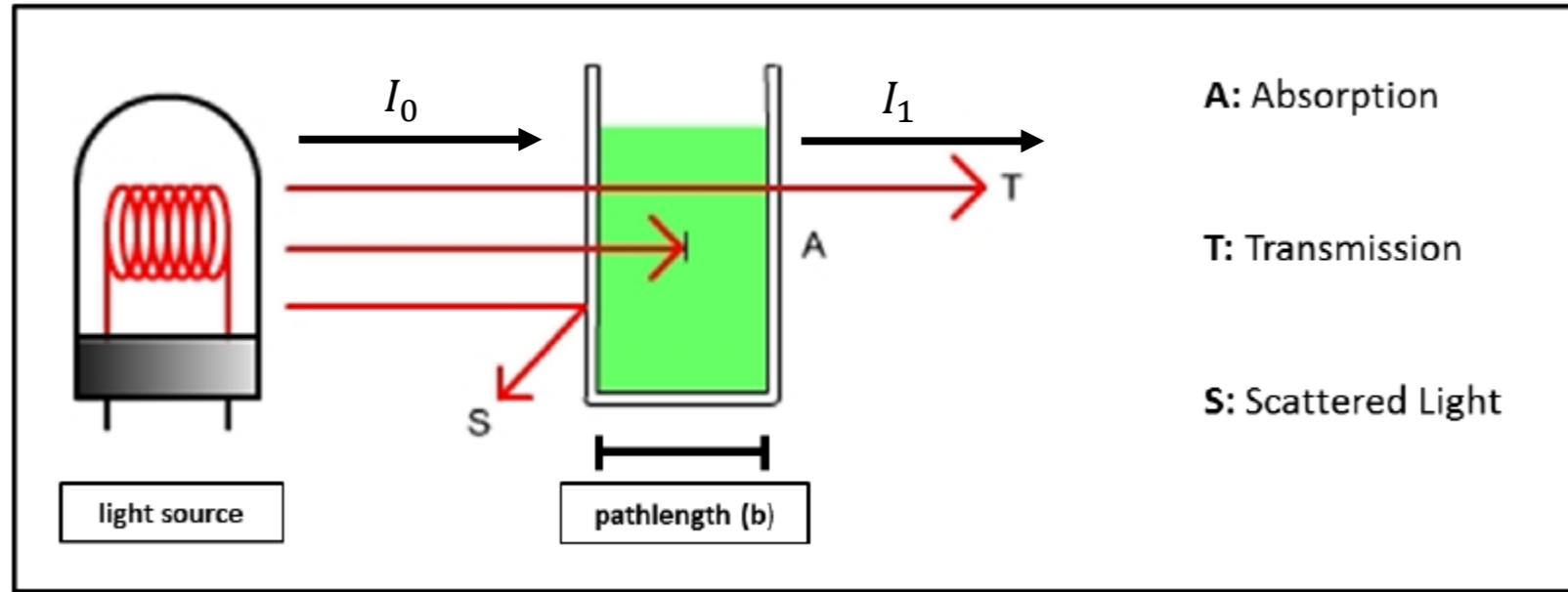
The screenshot shows the SkanIt 3.2 software interface. The 'Results' window is open, displaying a table of data. The 'Table' tab is selected, and the 'Export' button is highlighted with a red box. The table contains data for 8 rows (A-H) and 12 columns. The 'Export' button is located in the top right corner of the table area.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Cal_0001 1/2 1000 1e+03	Cal_0001 2/2 1000 1e+03	Un_0001 1/2 1:1 877	Un_0001 2/2 1:1 356								
B	Cal_0002 1/2 500 500	Cal_0002 2/2 500 500	Un_0002 1/2 1:1 910	Un_0002 2/2 1:1 273								
C	Cal_0003 1/2 250 250	Cal_0003 2/2 250 250	Un_0003 1/2 1:1 781	Un_0003 2/2 1:1 705								
D	Cal_0004 1/2 125 125	Cal_0004 2/2 125 125	Un_0004 1/2 1:1 265	Un_0004 2/2 1:1 439								
E	Cal_0005 1/2 62.5 62.5	Cal_0005 2/2 62.5 62.5	Un_0005 1/2 1:1 186	Un_0005 2/2 1:1 98.2								
F	Cal_0006 1/2 31.25 31.3	Cal_0006 2/2 31.25 31.3	Un_0006 1/2 1:1 572	Un_0006 2/2 1:1 403								
G			Un_0007 1/2 1:1 603	Un_0007 2/2 1:1 195								
H			Un_0008 1/2 1:1 63.5	Un_0008 2/2 1:1 102								

At the bottom of the interface, the status bar shows: Multiskan GO SIMULATOR - Connected, Disconnect, Target: 20.0°C, Plate: 20.4°C, Cuvette: 21.1°C, and admin.



Absorbance



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

I_1 = intensity of the radiation (light)
 I_0 = amount of light of the incident light beam

The Beer's law

$$A = \epsilon c l$$

A
 ϵ
 c
 l

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

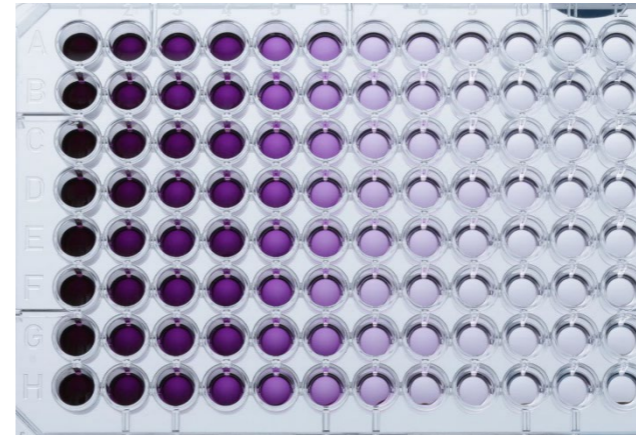


Multiskan GO

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

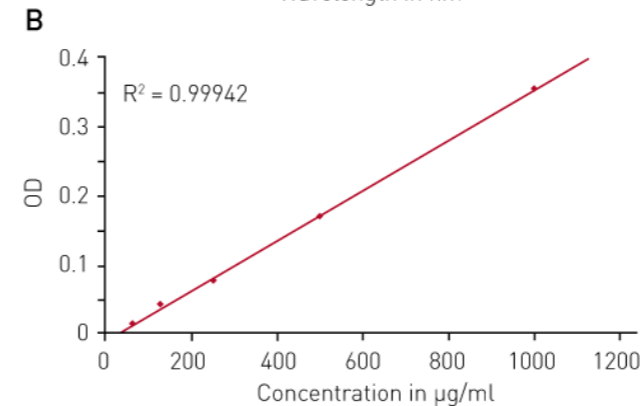
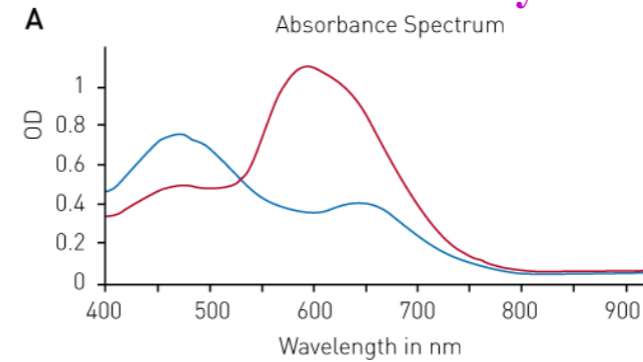


- ELISA
- Cell viability(MTT)
- BCA/Bradford
- DNA/Protein (260 nm / 280 nm)
- Microbial growth (OD 600 nm)

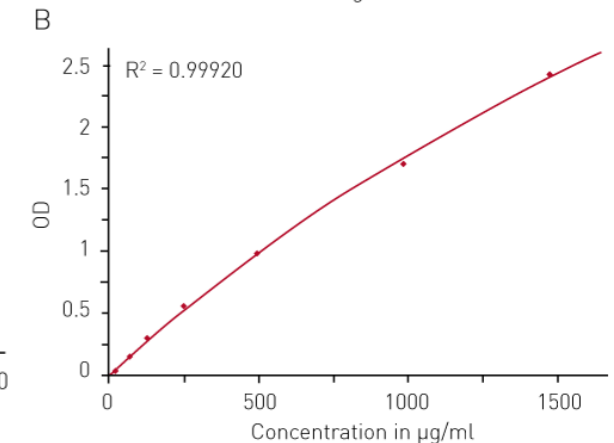
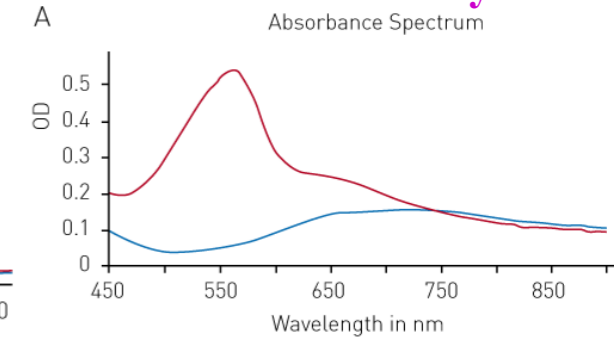


Cell viability(MTT Assay)

Bradford assay



BCA assay





~Thank You for Your Attention~