IMAPlate™ 5RC96

a Product from

Patented **I**ntelligent **M**ultifunctional **A**nalytical Technology

April 2012

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Introduction of IMAPlate™ 5RC96

http://www.youtube.com/IMAPlateTM

A standard 96-well plate formatted

disposable miniature multi-utility lab device

with Integrated functions of

Pipette, Test Tube, Cuvette ...

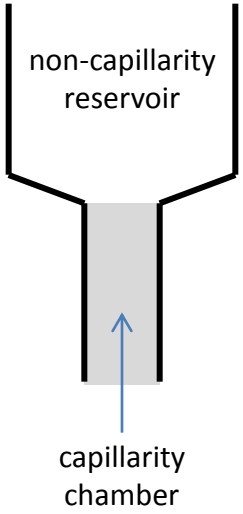
The world's first analytical platform

capable of manually performing parallel, high throughput

liquid transfer, reaction, analysis and assay.

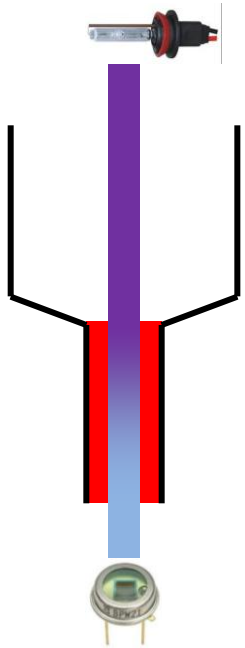
IMAPlate™ 5RC96 Working Principle – Capillarity

illustrations of unique self-dosed capillarity liquid handling (single unit)



The capillarity chamber is able to use capillary action to suck up quantitative amount of solution from the bottom opening and to keep the dosed solution in the capillarity chamber.

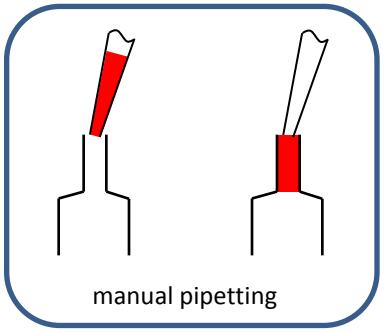
The solution can be collected into a 96-well plate by centrifugation or absorbed by using a filter paper .

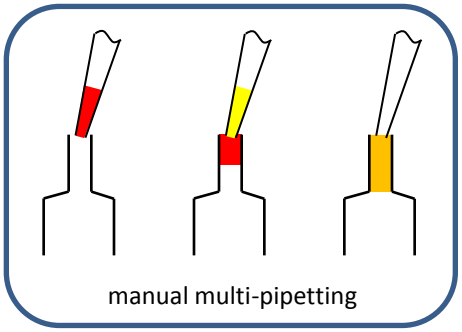


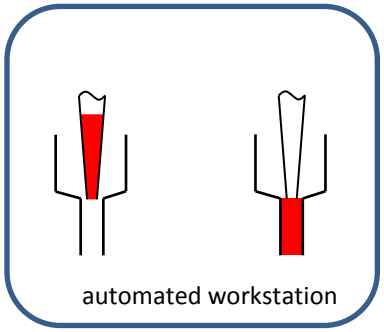
The solution can be measured in a 96-well plate reader.

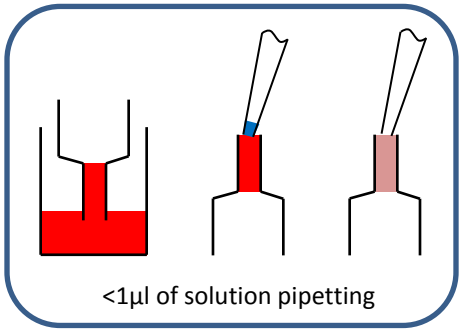
Illustrations of Pipetting Solution to IMAPlate™ 5RC96

methods for pipetting 1- 5µl or <1µl of solution

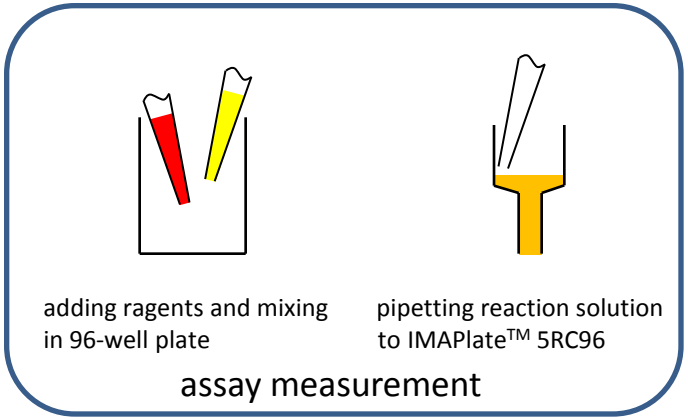

manual pipetting

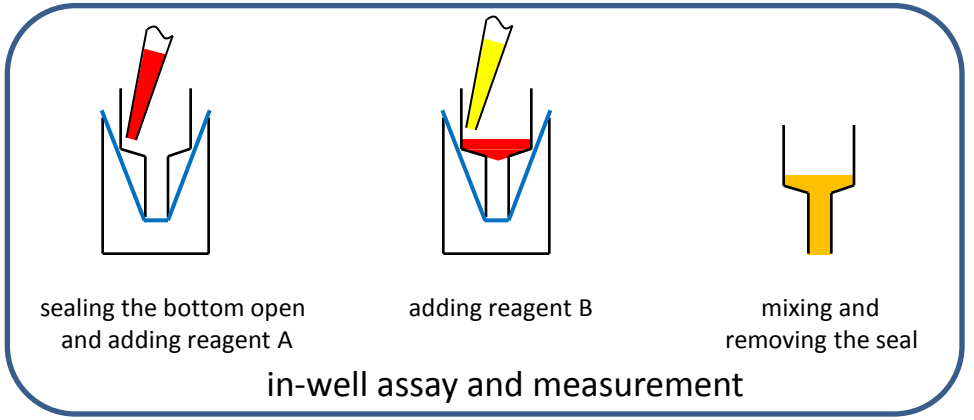

manual multi-pipetting


automated workstation


<1µl of solution pipetting

methods for pipetting 15-25µl of solution





IMAPlate™ 5RC96 Technical Data

comparison between IMAPlate™ 5RC96 and Nunc. MaxiSorp F96 plate

	IMAPlate™ 5RC96	Nunc. MaxiSorp
Plate Format	SBS 96-well format	SBS 96-well format
Well Dimension (Φ x H)	1.1mm x 5mm	6.9mm x 10.7mm
Total volume	5µl (or 50µl)	360µl
Working volume (V)	5µl (or 15 – 25µl)	100µl
Surface area of working volume (Sa)	$18mm^2$	$(60 + 34)mm^2$
Surface area to volume ratio (Sa/V)	$3.6mm^2/µl$	$0.94mm^2/µl$
Initial binding velocity d[AB]/dt (B ⇔ Sa/V)	3.8 k_{on}[A][B]	1 k_{on}[A][B]
Complex density [AB] (B ⇔ Sa/V)	3.8 Keq [A][B]	1 Keq [A][B]
Diffusion distance from center to wall (X)	0.55mm	3.5mm
Diffusion time ($X^2 = D_{dif} \bullet t$)	$0.3/D_{dif}$	$12/D_{dif}$
Light path length	5 or 7.5mm (5 or 25µl)	3mm (100µl)

Applications of IMAPlate™ 5RC96

- 96 channel pipette & replicator for simultaneous liquid transfer
  - sampling, dilution, spotting, inoculation, …
- 96 micro-cuvette for UV-Vis-IR full spectrum measurement with easy sample recovery option
  - DNA, RNA and protein quantification at UV, compound analysis at UV-Vis-IR, assay result readout to enhance sensitivity.
- 96 micro-well plate for reactions and assays
  - In-well homogeneous assays (e.g. miniature, high sensitivity assays)
  - In-well heterogeneous assays (e.g. miniature ELISA)
  - Solid phase enzymatic or binding assays for compound, ligand screen
  - 3D cell culture and cell based assays
  - Molecular biological assays
  - Proteomics applications
  - Multi-step solid phase chemical synthesis

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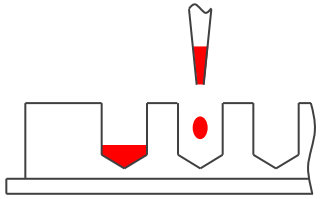
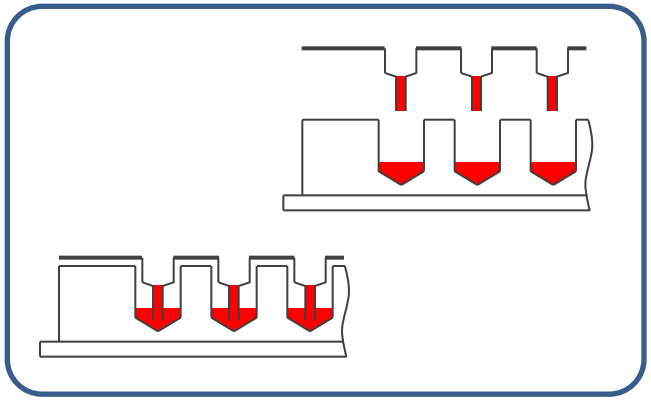
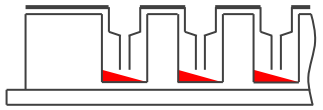
Illustrations of sampling, dilution, spotting, inoculation…



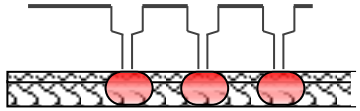
prepare a master plate



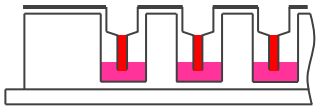
touch-load solution



spin to new plates for sampling, dilution and ...



spot on membrane for dot blot assay and ...



add to cell cultures in 96-well plate for making replication, screening compound and ...

Applications of IMAPlate™ 5RC96

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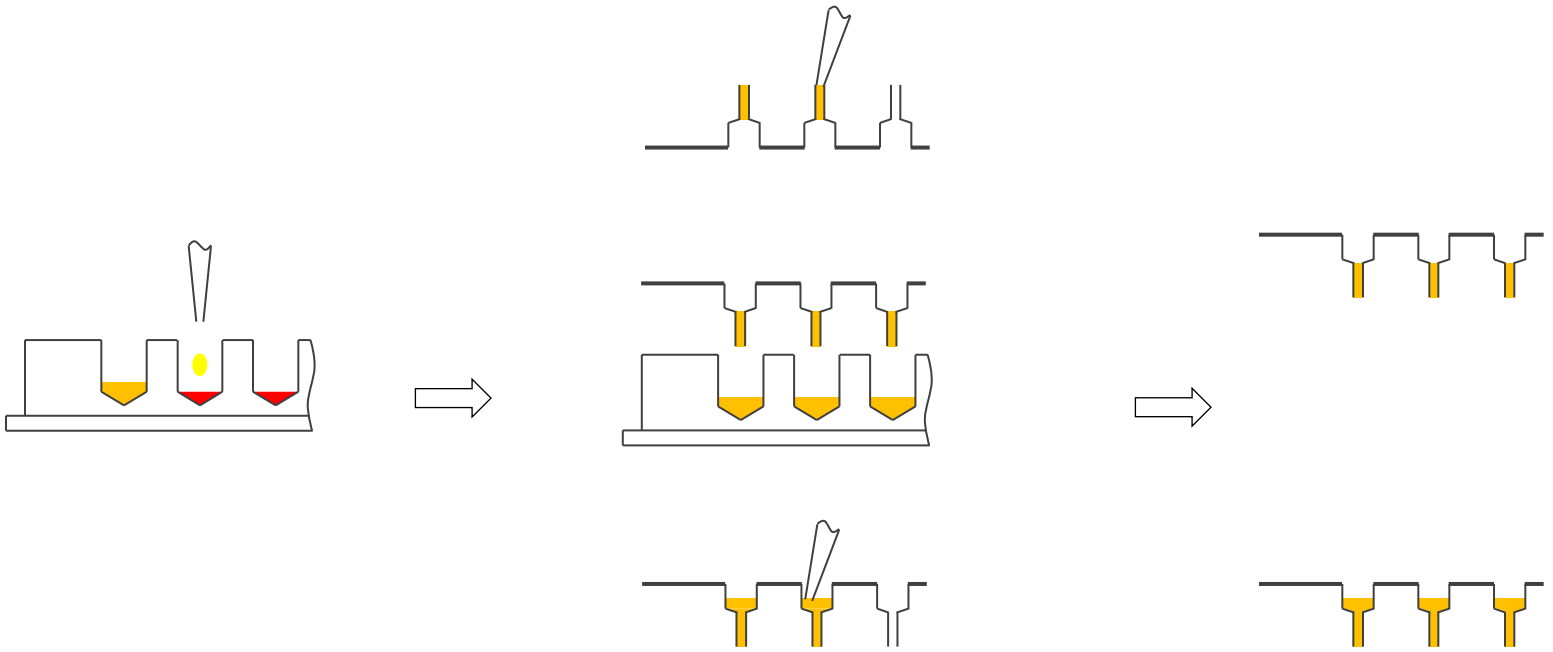
  - Multi-step solid phase chemical synthesis

Illustrations of assay in 96-well plate and measurement in IMAPlate™ 5RC96

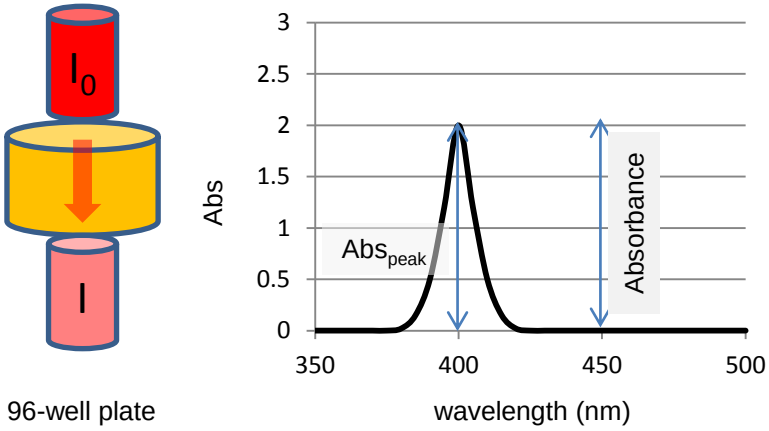


perform assay in 96-well palte

transfer assay solution to IMAPlate

measure IMAPlate in a reader

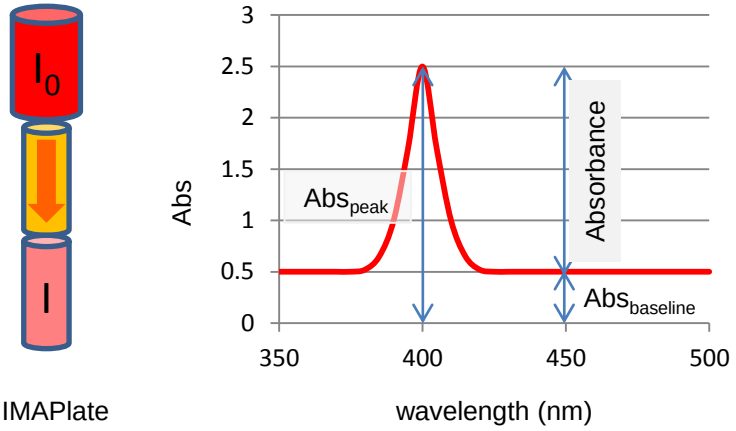
The use of two wavelength absorbance measurement to calculate the absorbance for IMAPlate™ 5RC96



96-well plate measurement

Absorbance = - logT = - log(I/I_0) **(1)**

The absorbance of the solution in 96-well palate is calculated according to the equation (1), where I_0 is the intensity of incident light and I is the intensity of transmitted light.



IMAPlate measurement

Due to small diameter of the capillarity chamber, the observed Abs is the sum of absorbance of the solution and blockage of incident light, which causes the baseline shifting upward. Assuming the blockage is the same over the spectrum, the absorbance can then be calculate by following equation (2).

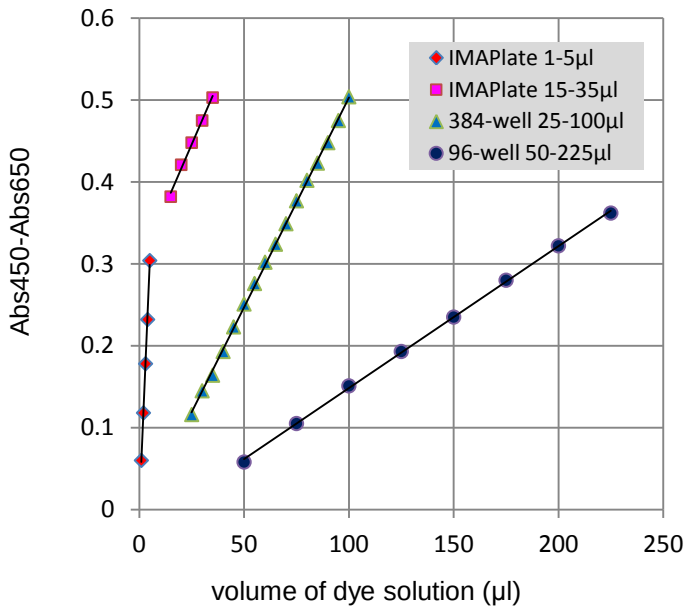
$Absorbance = Abs_{Peak} - Abs_{Baseline}$ **(2)**

High sensitivity for absorbance and fluorescence measurement resulted from long light path-length of IMAPlate™ 5RC96

- Absorbance of **5µl** dye solution in IMAPlate™ 5RC96 = **60µl** in 384-well plate = **175µl** in 96-well plate
- Fluorescence count of **5µl** fluorescein solution in IMAPlate™ 5RC96 = **85µl** in 96-well plate

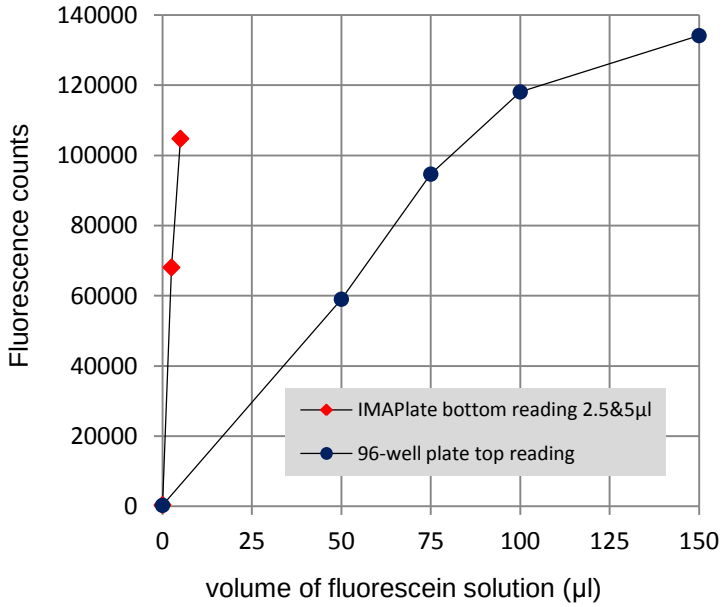
Absorbance vs Volume

(comparison of IMAPlate, 384- and 96-well plates)



Fluorescence vs Volume

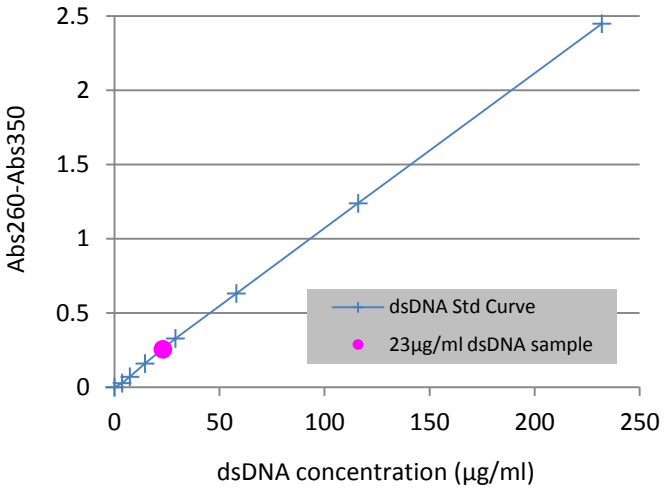
(comparison of IMAPlate and 96-well plates)

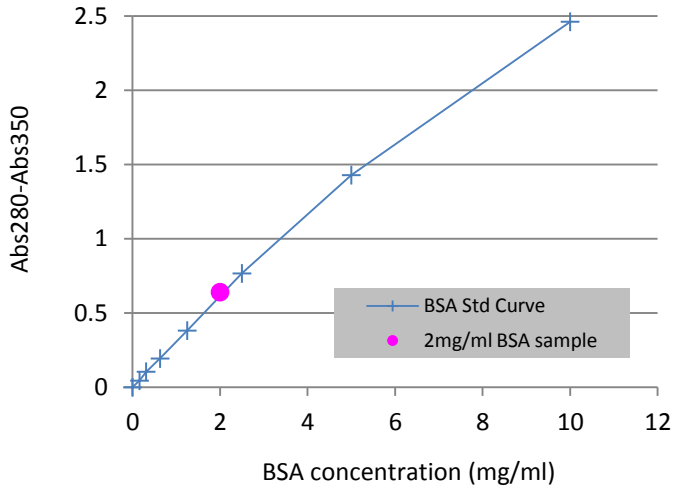


** Data obtained from PerkinElmer EnSpire® Multimode Plate Reader.*

High-throughput small sample volume quantification by touch-loading from a 96-well plate

DNA and protein measurement at UV





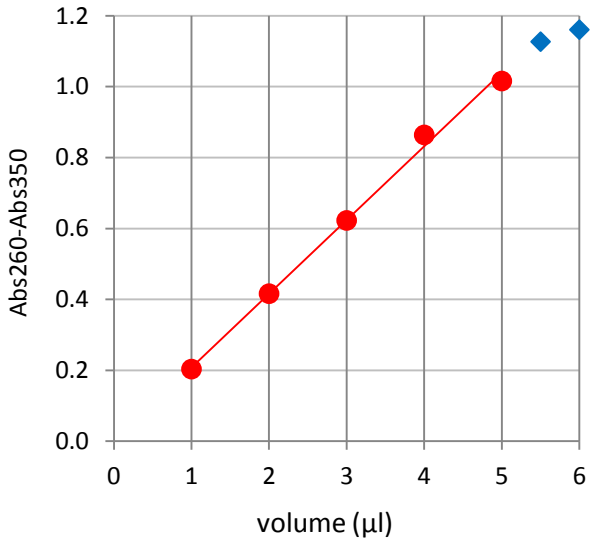
DNA (µg/ml)	Abs	SD	CV%
0	0.000	0.001	
3.6	0.028	0.003	10.9
7.3	0.071	0.003	4.3
14.5	0.160	0.001	0.6
29	0.329	0.004	1.2
58	0.632	0.006	1.0
116	1.239	0.015	1.2
232	2.449	0.004	0.2
23	0.256	0.011	4.3

BSA (mg/ml)	Abs	SD	CV%
0	0.000	0.001	
0.16	0.045	0.001	1.6
0.31	0.105	0.004	3.4
0.63	0.194	0.006	2.9
1.25	0.382	0.017	4.4
2.5	0.767	0.041	5.4
5	1.429	0.079	5.5
10	2.462	0.003	0.1
2	0.641	0.008	1.3

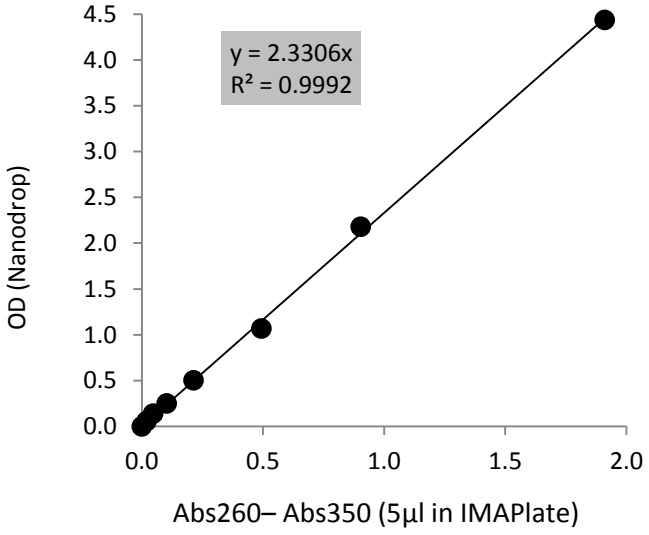
Good correlation with other measuremnt method

- DNA measurement by pipetting to IMAPlate
  - ❖ linear increase in Absorbance vs volume in the range of 1 to 5.5µl.
  - ❖ good correlation with nanodrop.

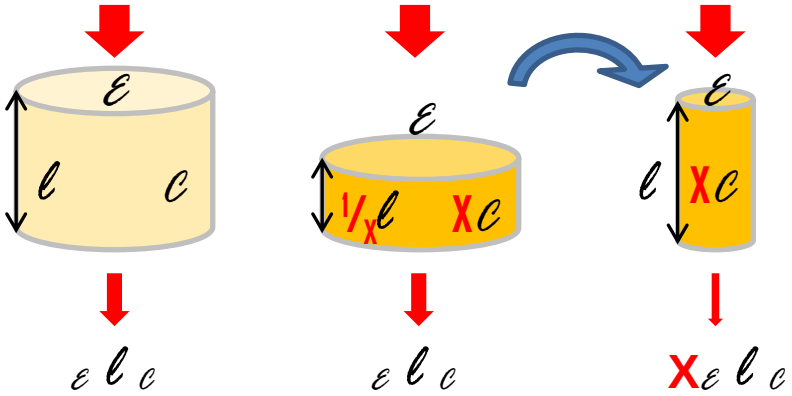
Absorbance vs Volume



Correlation
(Nanodrop and IMAPlate)



To increase sensitivity of ELISA by reducing substrate volume and transferring the solution to IMAPlate™ 5RC96 for measurement

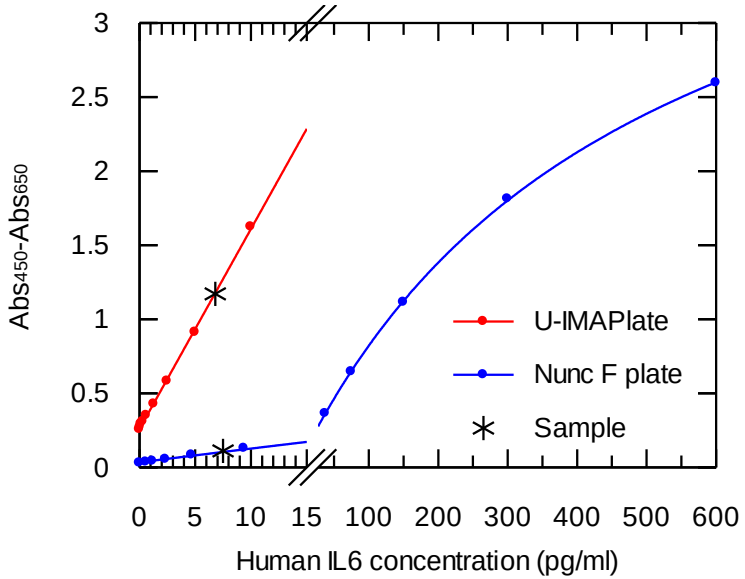


Abs = $\varepsilon \ell c$

- According to Beer-Lambert Law, light absorbance is the multiplication of absorption coefficient (ε), path length (ℓ) and concentration (c).
- With same amound of enzyme in the well, the less volume of substrate solution, the higher concentration of colored product and the shorter path length for colored solution.
- Abs will not increase when using less substrate and measuring in the same well because the shorter path length cancels out the higher concentration of colored product.
- Once the colored solution is transferred to IMAPlate for measurement, the Abs increases due to the the higher concentration of colored product and the longer path-length of IMAPlate.

Human IL6 ELISA

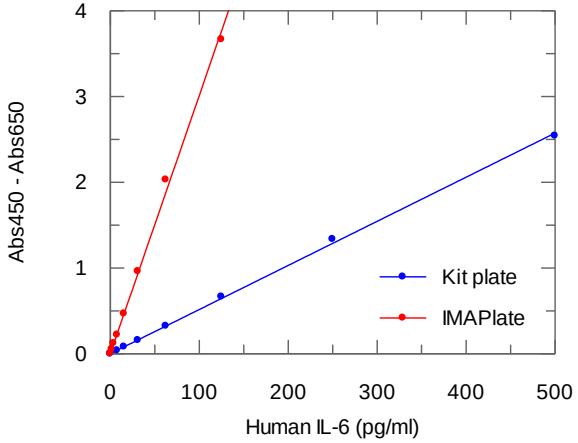
(15µl TMB & 5µl stop solution with touch-loading)



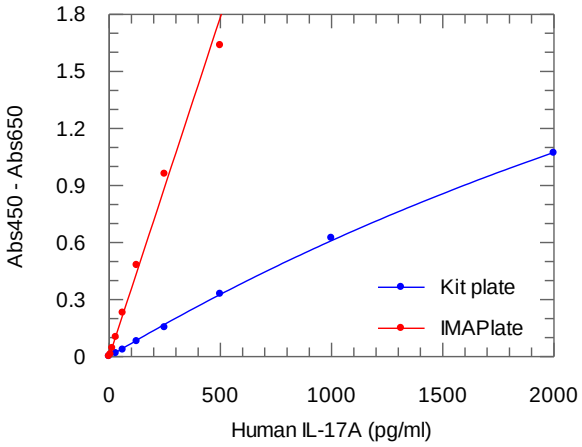
Spies P. et al. A simple approach to improve the sensitivity of enzyme-linked immunosorbent assay: Using the IMAPlate 5RC96 for result readout, Analytical Biochemistry, 397 (2010): 48-55.

To increase sensitivity for commercial ELISA kit (pre-coated plate)

LEGEND MAX™ Human IL6 ELISA



LEGEND MAX™ Human IL17A ELISA



Procedure

- Prepare a set of extanded standard with additional several lower concentration points.
- Perform the ELISA according to the protocol in the kit **till to the step before the addition of substrate solution**. Then follow the procedure below.

1) *Add 40µl of TMB solution instead of 100µl to the wells of ELISA kit plate.*
2) *Shake the plate on the plate shaker with 700-800 rpm for 15-20min.*
3) *Pipette 10µl of the stop sulotion to the wells to stop the reaction.*
4) *Transfer 40µl of the reaction mixture to the non-capillarity reservoir of the IMAPlate.*
5) *Tap the IMAPlate several times horezontally to facilliate solution go to the capillarity chamber.*
6) *Measure the IMAPlate at 450nm and 650nm in a plate reader.*
7) *Calculate the true Abs by substrating Abs450 by Abs650 for plotting.*

Applications of IMAPlate™ 5RC96

- 96 channel pipette & replicator for simultaneous liquid transfer
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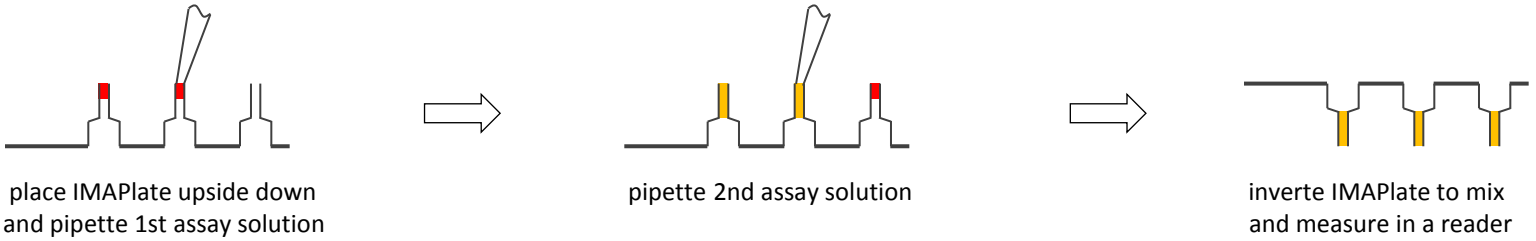
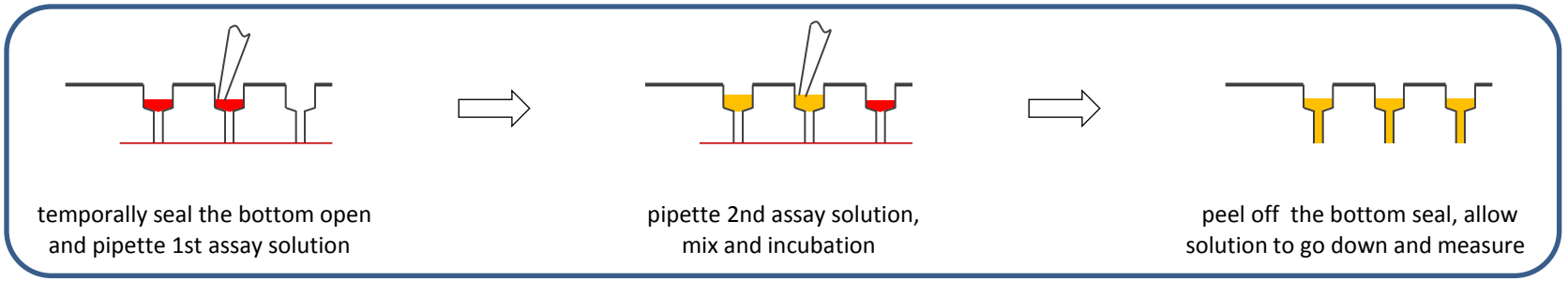
Illustrations of in-well homogeneous assay

5µl low volume assay with add-mix-meaure protocol



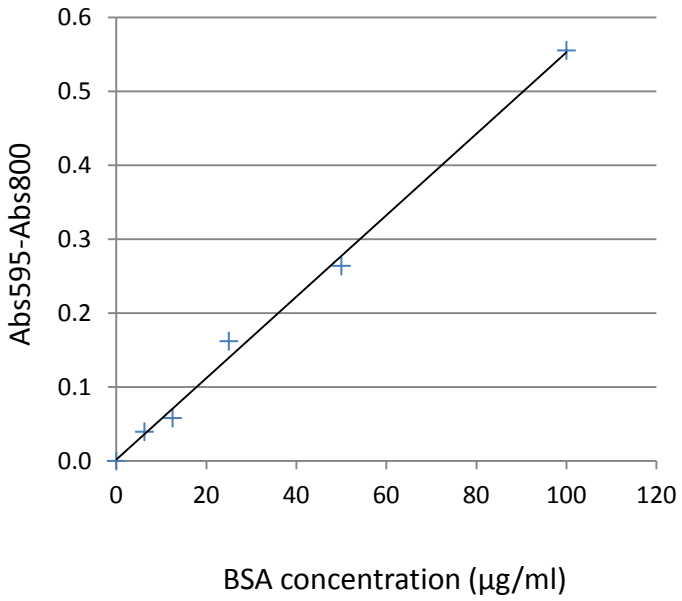
15-25µl macro volume assay with add-mix-measure protocol with incubation



In-well miniature protein quantification assay

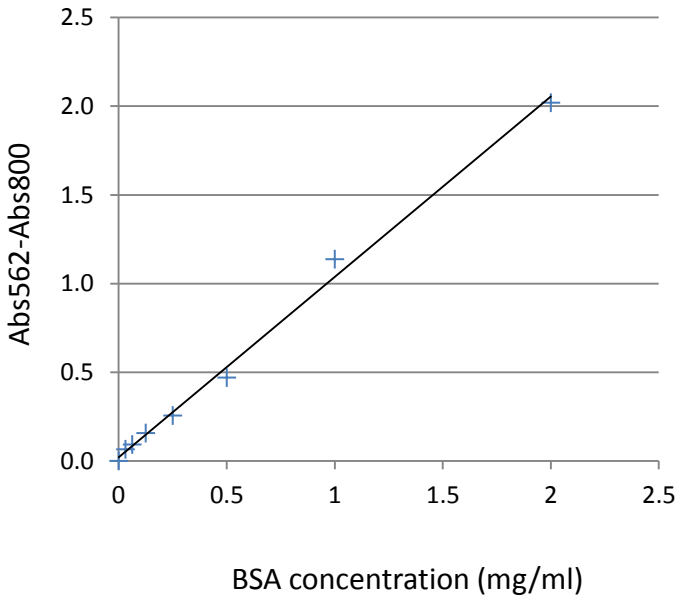
5µl Bradford assay

4µl of protein and 1µl of Bradford reagent mixed in the capillarity chamber by inverting the IMAPlate and measured immediately.



25µl BCA assay

2.5µl of protein and 22.5µl BCA working reagent incubated 30min at 37°C on a shaker in the non-capillarity reservoir of a parafilm sealed IMAPlate. Peeled off the parafilm and measured.

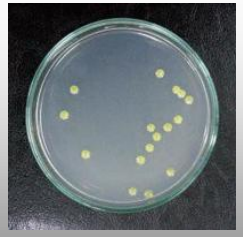


In-well miniature enzyme assay (15-25µl)

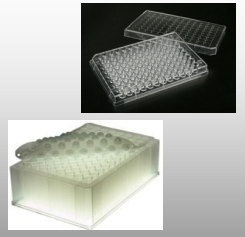
- e.g. Candida Antarctica Lipase B assay in IMAPlate for rapid colony screening

*M. A. Sciotti et. al. IMAPlate Based Miniature, High Sensitive, Rapid Screening Method for Detecting Bioengineered, Secreted Lipase Activities in Yeast Expression Systems. *CHIMIA, Volume 64, No. 11, November 2010, Pages 789-792*

- ✓ only 1/10 assay volume of 96-well plate
- ✓ higher sensitivity
- ✓ reducing assay cost
- ✓ minimizing upstream processing scale
- ✓ saving culture medium and reagents
- ✓ short screening cycle time

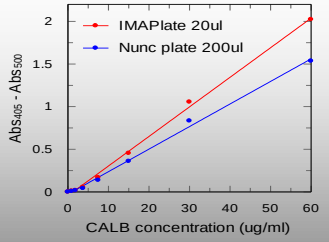


pick up individual whole clony



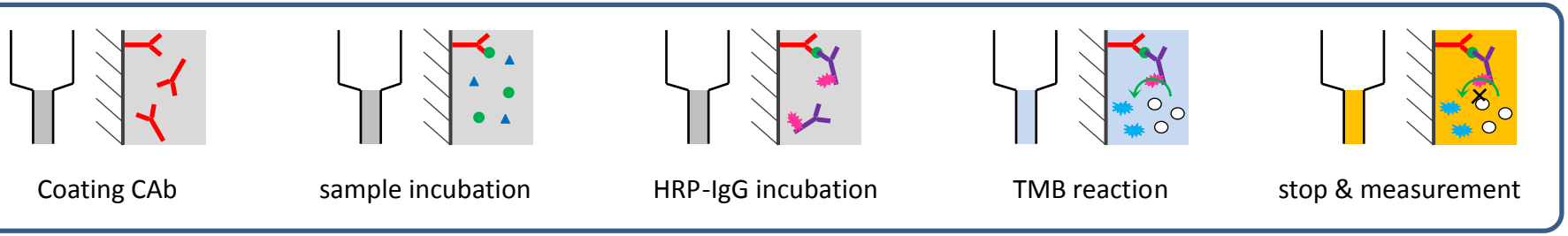
inoculate to 96-well plate for protein expression

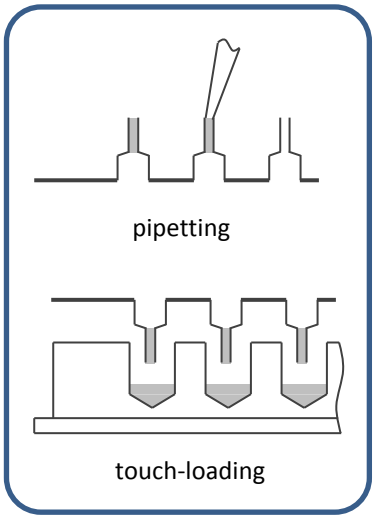
test enzyme activity in medium and lysate



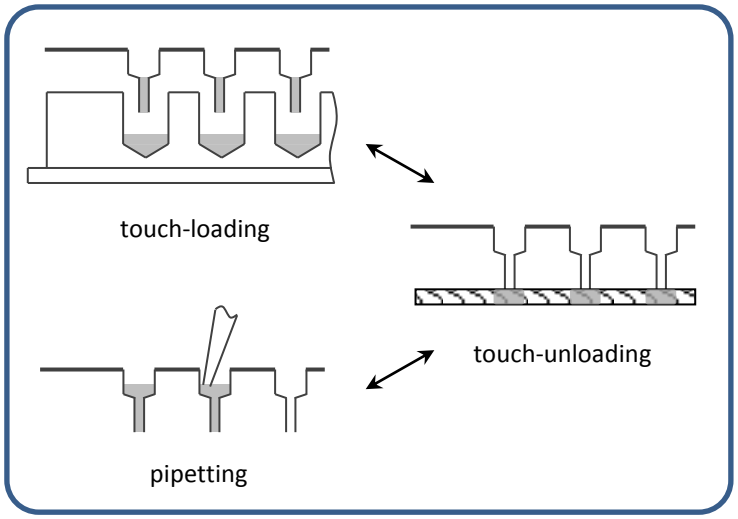
plot Std curve for data analysis

Illustrations of in-well miniature heterogeneous assay – ELISA

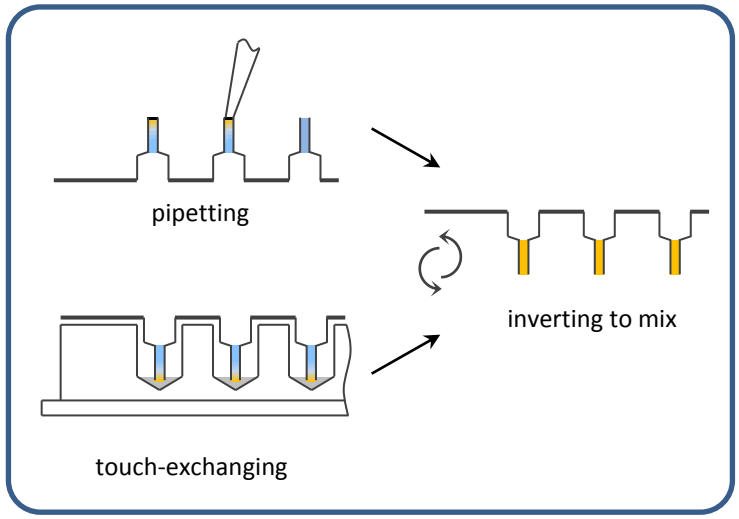




method for adding reagents and sample



method for washing



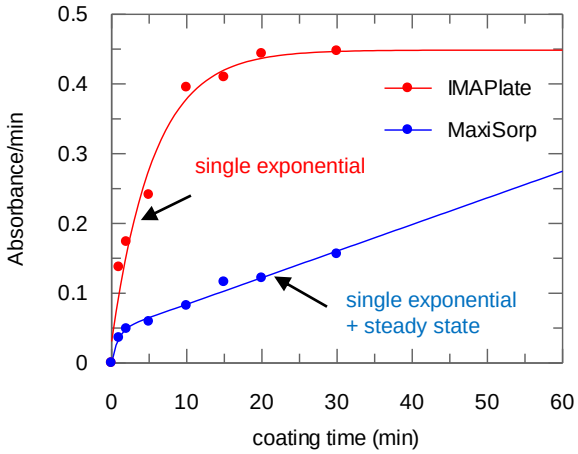
method for stopping reaction

large surface area to volume ratio and short diffusion distance leading to the increase in initial binding velocity and short time to reach equilibrium

- protein binding shown to obey single exponential kinetics
- formation of antigen antibody complex shown single exponential behavior as well

Time Course of

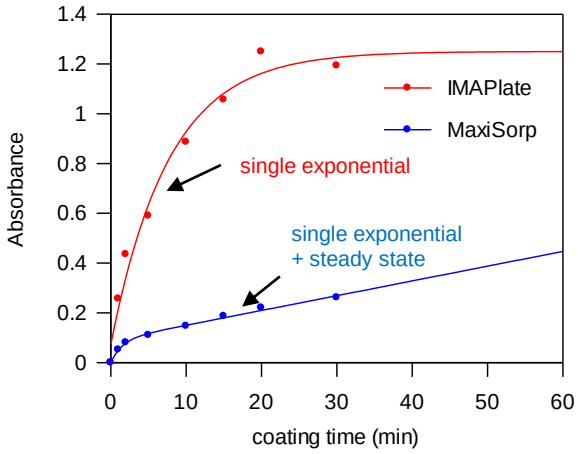
protein binding



1) Coating: IgG-HRP in 1 µg/ml BSA (diluted from 15 mg/ml BSA containing IgG-HRP)

2) TMB reaction: 5min for IMAPlate and 15 min for Nunc plate

IgG binding

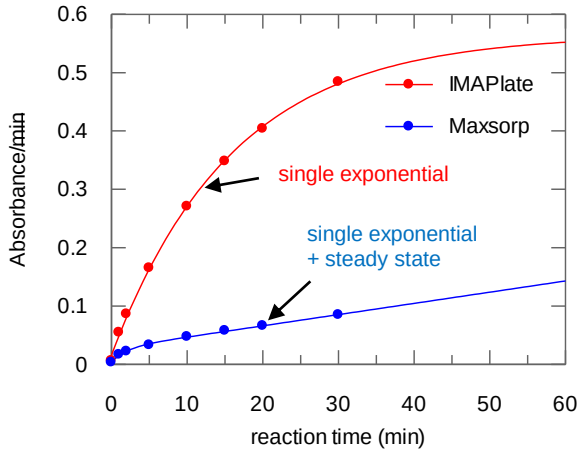


1) Coating: 10 ng/ml GxR-IgG

2) Reaction: 1:20K R-IgG-HRP, 30min

3) TMB reaction: 15 min

complex formation



1) Coating: 2 µg/ml GxR-IgG, 30 min

2) Reaction: 1:150K R-IgG-HRP

3) TMB reaction: 5min for IMAPlate and 15 min for Nunc plate

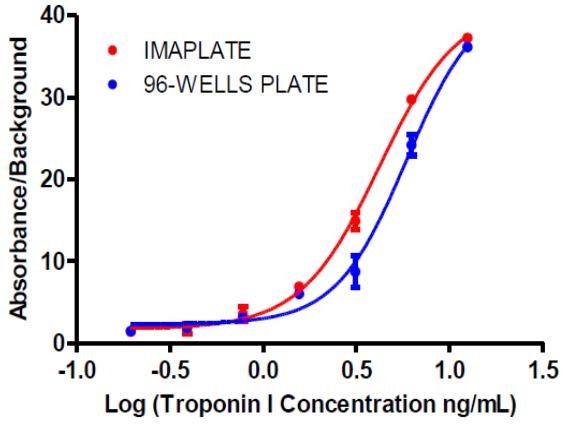
In-well miniature ELISA

- small sample volume
- higher sensitivity
- short time to result
- less comsumption of reagents

Rapid Miniature Troponin I ELISA

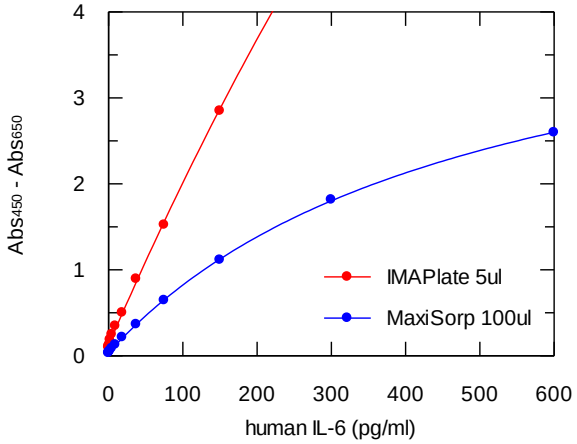
IMAPlate: <**3**hrs incl. coating

96-well plate: **7**hrs + **ON** coating



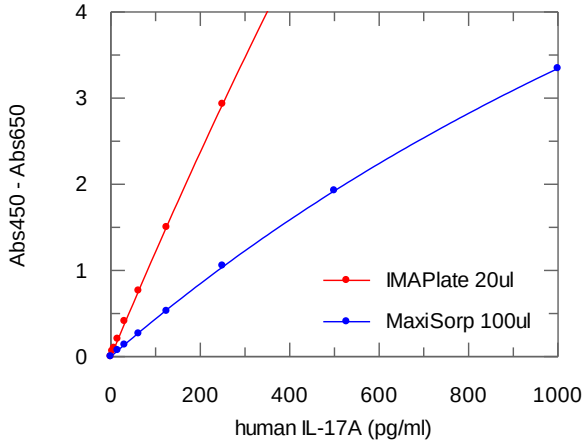
from PerkinElmer Application Note: Using the EnSpire Multimode Plate Reader to Measure IMAPlate-Based Rapid Miniature ELISA for the Quantification of Troponin I

Human IL-6 ELISA



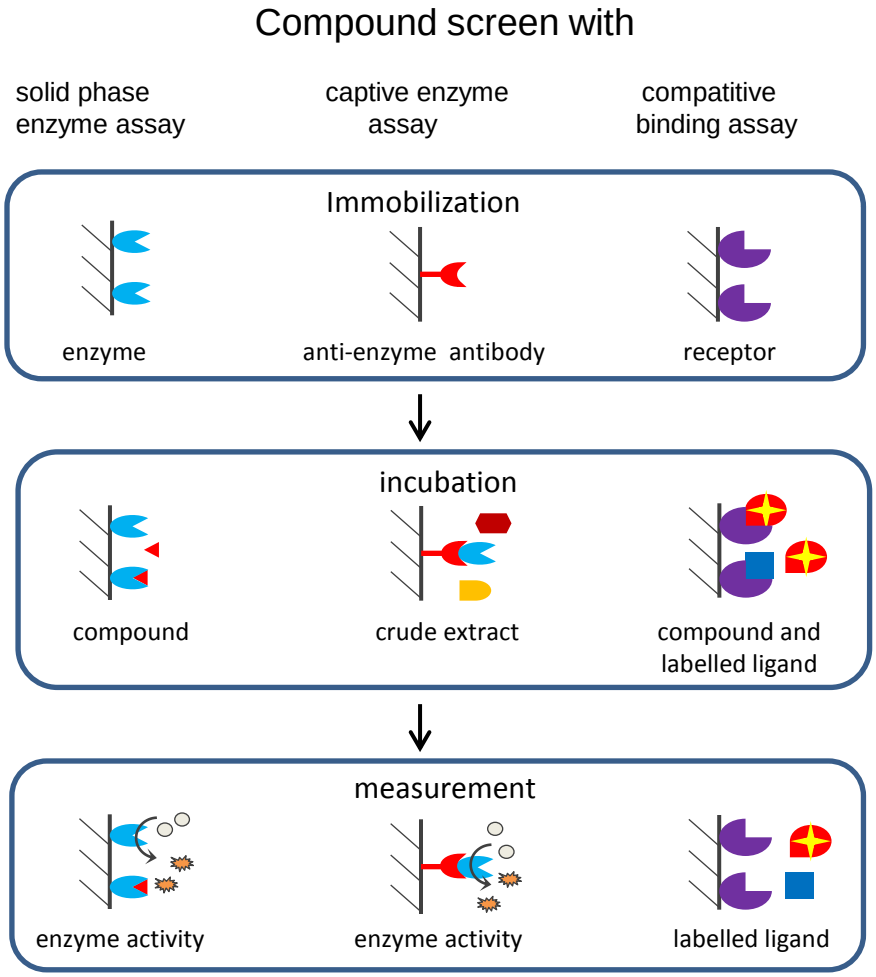
DuoSet kit from R&D systems: 1) coating O.N. 2) 1st incubation 120min 3) 2nd incubation 120min 4) SA-HRP 20min and 5) TMB 20min.

Human IL-17A ELISA



MAX™ Deluxe Kit from Biolegend: 1) coating O.N. 2) 1st incubation 120min 3) 2nd incubation 60min 4) SA-HRP 30min and 5) TMB 15min

Illustrations of in-well miniature heterogeneous assay – assays for compound screen



Advantages

- ❑ easy wash procedure
- ❑ solid phase enzyme assay
  - able to eliminate the interference of colored compound on assay measurement
  - possible to reuse the same coated plate several times when enzyme is stable
- ❑ captive enzyme assay
  - reducing other enzymes interference
  - minimizing extract caused matrix effect for the assay

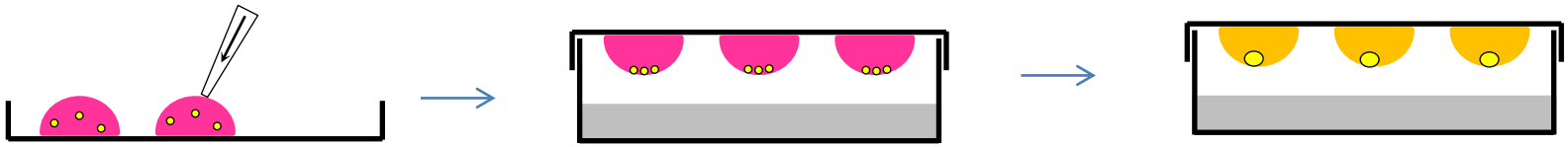
Why switching from 2D cell culture to 3D cell culture?

Drug candidates showing promise in monolayer 2D cell cultures often fail in later stages of drug development.

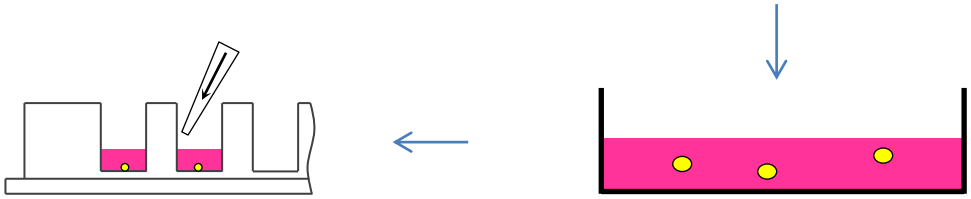
- cells altering the in vivo morphological and physiological characteristics when cultured in 2D
- constrained cell signaling mechanisms between adjacent cells due to limited cell-cell interactions in 2D
- 3D spheroids having similar cell-cell interactions like tissues in vivo
- 3D spheroid displaying very similar gene expression pattern of clinical specimens

Illustration of hanging drop 3cell culture in Petri dish

(limitations: medium change and spheroid transfer)



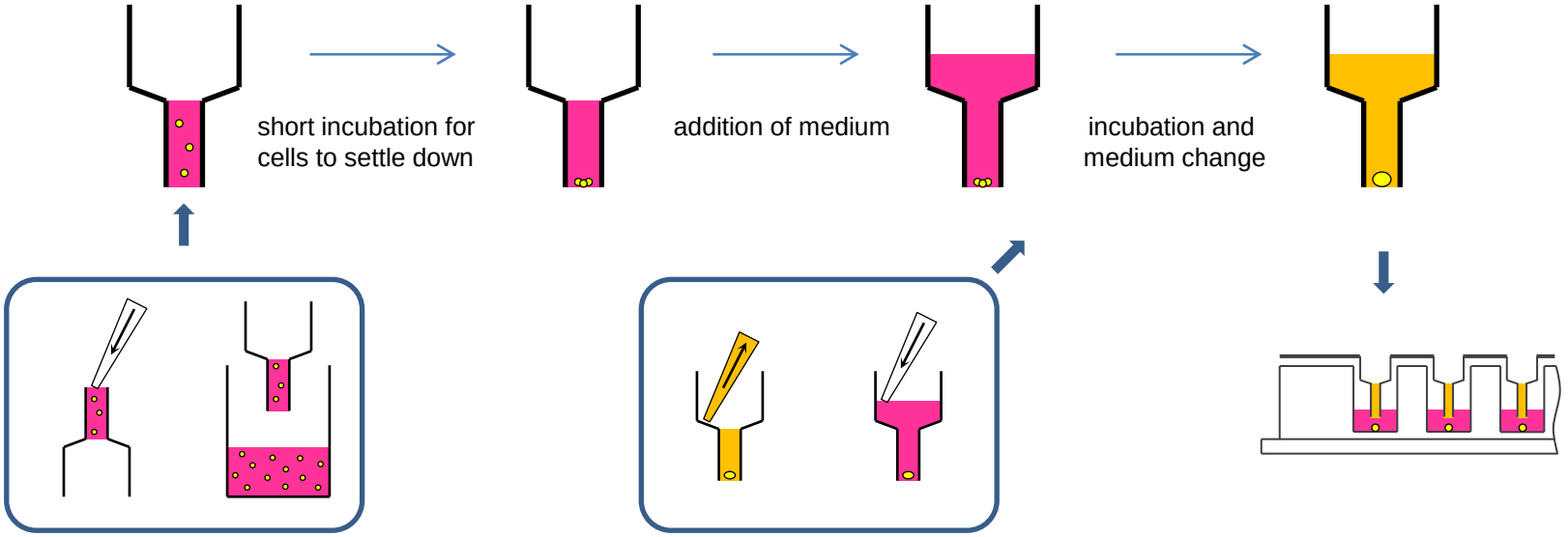
pipette cell suspension on the cover of Petri dish and culture the cells to form spheroids

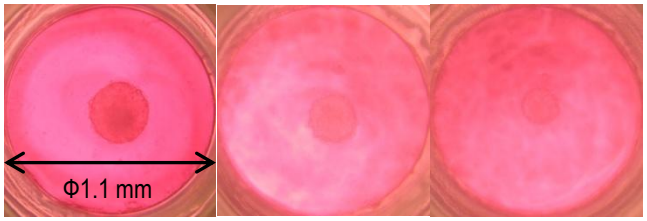


transfer to 96-well plate

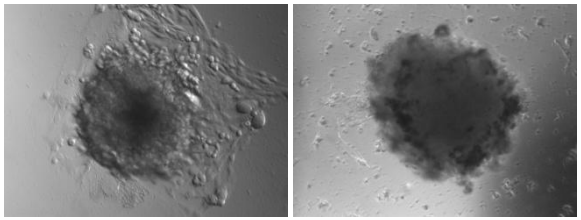
wash off the spheroids

Illustrations of the preparation of spheroid micro-tissue and embryoid body in IMAPlate™ 5RC96

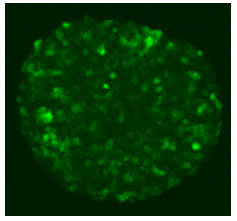




U87 spheroid in IMAPlate™ 5RC96
(2 days after seeding 5K, 2.5K and 1K cells: right to left)



LN319 spheroid in assay plate
(2 days after exposure to compound)



HEK Spheroid
(expressing GFP)

Advantages of using IMAPlate^{TM} 5RC96 as a “hanging drop” cell culture device for 3D cell culture

1. easy to change cell culture medium
2. no risk of loss spheroid when aspiring the medium from the non-capillarity reservoir
3. rapid spheroid transfer from IMAPlate to 96- & 384-well plate
4. no harsh operating procedure for spheroid transfer
5. able to prepare and test spheroids in the same IMAPlate
6. capable of preparing large quantity of spheroids
7. easy to estimate the size of spheroids
8. cells readily to form spheroids in small capillarity chamber
9. possible to use a shaker to facilitate the formation of spheroids

Summary

Features of IMAPlate

- Miniature:
  - ✓ minimizing sample consumption
  - ✓ reducing assay cost
- Unique and flexible liquid handling
  - ✓ simultaneous filling/emptying samples
  - ✓ retaining pipette liquid handling options
  - ✓ avoiding air gap formation with open end
- Improvement for reaction and analysis
  - ✓ increasing sensitivity for measurement
  - ✓ rapid adsorption and binding
  - ✓ short time for reaching equilibrium
  - ✓ UV-Vis-IR full spectrum measurement
- Compatible with 96-well plate readers and automated liquid handling systems
  - ✓ no special equipments required

Benefits of using IMAPlate

- ✓ able to obtain multiparameter from limited amount of sample
- ✓ minimizing the scale of upstream process such as cell culture, fermantation, and protein purification
- ✓ no killing for small animal study and using less aninmal
- ✓ able to follow the same aninmal at time points and reducing variation of individual difference
- ✓ improving public images on animal research and industrial waste production (green technology)
- ✓ manual high-throughput
- ✓ short time to result
- ✓ reducing time for screening cycle
- ✓ enhancing lab productivity

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