



IMAPlate™ 5RC96 Application Note

Miniaturized *Enzyme-Linked ImmunoSorbent Assay*

Introduction

Enzyme linked immunosorbent assay (ELISA), as a simple, sensitive, specific and economical method for qualification and quantification of a particular molecule (e.g. protein, peptide or small molecule) in a sample, is routinely used in life sciences, health care, and many other different industries.

Although it is developed in late 70's, the ELISA has almost kept its original assay format; the assay is performed in a 96-well plate with a working volume of 100µl or 200µl. Attempt to reduce the reaction volume of the ELISA by using 384-well plate with a working volume of 20µl or 50µl has been reported successful. But technical difficulties in liquid handling hampered such kind of approaches being used.

IMAPlate™ 5RC96 is the world's first miniaturized analytical platform, capable of manually performing high-throughput liquid transfer, analysis, reaction and assay. Its unique liquid handling concept has resolved the technical difficulties for transferring tiny amount of solution. Up to 96 individual samples or solution can simultaneously be transferred in and out of the 5 µl reaction chambers of the IMAPlate™ 5RC96 by touch-loading or touch-unloading procedure. Therefore, the processing of each step for ELISA is in parallel and high-throughput. This unique liquid handling concept especially eases the tedious washing steps of the ELISA.

The use of IMAPlate™ 5RC96 for ELISA would bring scientists many benefits such as:

- minimizing the consumption of delicate samples and reagents - 5 µl
- reducing time to result by at least half
- high productivity
- cost effectiveness
- user friendly
- benefit environment - producing less biological and chemical wasters

Experimental

Reagents and Materials

- IMAPlate™ 5RC96 start kit
- Human IL-6 DuoSet ELISA Development kit
- U-bottomed 96-well plates
- Pasture pipette and/or pipettes
- Microwell plate reader

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Miniaturized ELISA

High-throughput Protocol

Plate Preparation

1. Dilute the Capture Antibody to the working concentration (2 µg/ml) in PBS according to the protocol provided by the manufacturer. Add one drop (between 20µl to 50µl) of the diluted Capture Antibody to each well of the U-bottomed 96-well plate by a pasture pipette or a pipette. Immediately coat the IMAPlate™ 5RC96 plate(s) by touch-loading. Incubate 60 minutes in a humidity environment at room temperature.
2. Touch-unload the reaction chambers and wash with Wash Buffer by touch-loading and touch-unloading Wash Buffer three times.
3. Block IMAPlate™ 5RC96 by touch-loading of Reagent Diluent (1mg/ml BSA in PBS) to each reaction chamber. Incubate 10 minutes in a humidity environment at room temperature.
4. Wash three times as the step 2. The IMAPlate™ 5RC96 plates are now ready for sample addition.

Assay Procedure

1. Touch-load sample or standard in Reagent Diluent from the U-bottomed 96-well plate, which is prepared in advance by adding a drop of the samples or the standards to the appropriate wells. Incubate 60 minutes in the humidity chamber at room temperature.
2. Wash three times as the step 2 of Plate Preparation.
3. Touch-load the Detection Antibody diluted in Reagent Diluent from a U-bottomed 96-well plate. Incubate 60 minutes in the humidity chamber at room temperature.
4. Wash three times as the step 2 of Plate Preparation.
5. Touch-load 1:200 diluted Streptavidin-HRP solution from a U-bottomed 96-well plate. Incubate 15 minutes in the humidity chamber at room temperature.
6. Wash five times as the step 2 of Plate Preparation.
7. Touch-load Substrate Solution. Incubate 5 - 10 minutes at room temperature.
8. Place the IMAPlate 5RC96 on a U-bottomed 96-well plate containing 15µl of stop solution (2 - 5M H₂SO₄) in each well. Make sure the bottom openings are contacted with the stop solution. After touch-exchange 30 seconds, slowly remove the IMAPlate 5RC96 from the U-bottomed 96-well plate and gently invert several times until the blue colored TMB substrate solution turning to yellow.
9. Immediately determine the **true absorbance** of each reaction chamber by a two-wavelength measurement: measure both peak absorbance at 450nm and the baseline absorbance at 650nm (any wavelength between 550nm to 700nm can be used) and subtract the peak absorbance from the correspondent baseline absorbance to get true absorbance ($Abs_{true} = Abs_{450nm} - Abs_{650nm}$). The true absorbance should be used for the calculation and plotting. If the reader has spectral scan mode, it is recommended to use the spectral measurement setting for the two-wavelength measurement e.g. starting at 450nm and ending at 650nm with a step of 200nm.

Sample & Reagent Saving Protocol

The plate preparation and the assay procedure in this Sample & Reagent Saving Protocol basically follow the same protocol as the above High-throughput Protocol except of the way to load reaction chamber. When it needs to save the samples or reagents, the reaction chambers are loaded by a pipette instead of by the touch-loading. It is recommended to turn the IMAPlate™ 5RC96 upside down and to use the reverse pipetting technique to add 5µl of samples or reagents into the reaction chambers through the bottom openings. **Extra care should be taken on the sample and standard orientation.** In order to avoid the orientation mistakes, mark the backside of the IMAPlate™ 5RC96.

Miniaturized ELISA

Results and Discussion

Figure 1 shows a typical plot of the data set of human IL-6 standards from an ELISA that was performed in IMAPlate™ 5RC96 with a high-throughput protocol, and a fitting curve of the standards with one-site binding mode. The standards were very well distributed around the fitting curve and the CV% of the IL-6 standards was usually less than 10% in triplicates (typically around 5%). When the same concentration of all the reagents was used to perform ELISA on conventional 96 well plate and IMAPlate™ 5RC96, the slope of the standard curve markedly increased with IMAPlate™ 5RC96. Therefore, the sensitivity was correspondently increased even that the time for reactions in IMAPlate™ 5RC96 was reduced to half.

It has to be pointed out that, when the sensitivity increases, the detection range may be narrowed due to the overflow of the absorbance for higher concentration standards (each plate reader has a certain absorbance detection range). Using a plate reader with a wide detection range may avoid the overflow for higher concentration standards. However, the missing peak absorbance data at 450nm can always be derived from the data set measured at a less sensitive, off-peak wavelength (e.g. at 485nm) by the linearity relationship between the data set at peak wavelength and the one at off-peak wavelength (Fig.2). Of course, further shortening reaction time for the color development or reducing Streptavidin-HRP concentration are possible alternative ways to avoid the overflow if the sensitivity does not need to be increased.

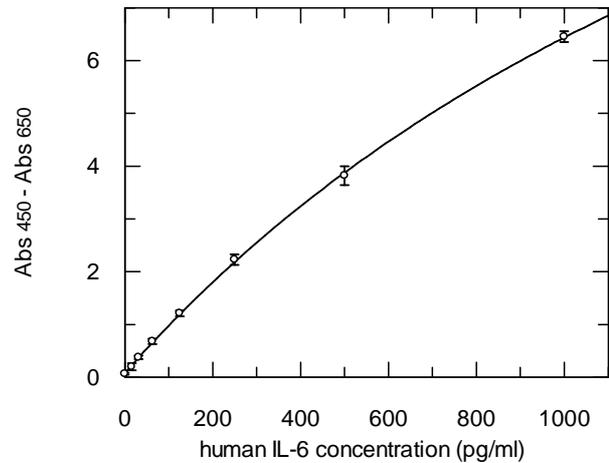


Fig. 1 Standard curve of an ELISA performed in IMAPlate 5RC96 using a human IL-6 DuoSet Kit

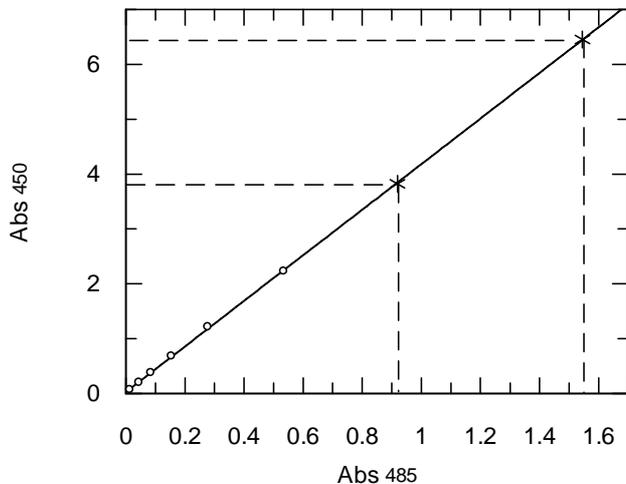


Fig.2. Deriving the missing data at 450nm by using linearity relationship between the peak data set at 450nm and the off-peak data set at 485nm

Conclusion

Using the IMAPlate™ 5RC96 for the ELISA can dramatically reduce the consumption of the sample and reagents. The time to results for a typical ELISA can be cut at least to half. The sensitivity is also increased. The unique touch-loading and touch-unloading procedure provides a parallel, high-throughput liquid transfer and especially eases the wash steps. Besides, the self-dosed solution up-taking may not need to use low volume precision pipette and can save the pipette tips. The IMAPlate™ 5RC96 is a user friendly, robust, miniaturized, high-throughput lab device for performing miniaturized ELISA.

NCL New Concept Lab GmbH

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Miniaturized ELISA

Ordering information:

Catalog No:	Article	Contents
NCL-STK-001	IMAPlate™ 5RC96 Start Kit	5 IMAPlate™ 5RC96 plates 1 reader adaptor (adjustable) 1 tool for adaptor adjustment 1 data sheet
NCL-P5W-002	White IMAPlate™ 5RC96	5 plates / box
NCL-P5B-004	Black IMAPlate™ 5RC96	5 plates / box
NCL-P5T-006	Transparent clear IMAPlate™ 5RC96	5 plates / box
NCL-P5Y-008	Transparent yellow IMAPlate™ 5RC96	5 plates / box

Products selection:

IMAPlate™ 5RC96	Liquid transfer	Absorbance measurement	Fluorescence measurement	Reaction
White	√	UV-Vis-IR	-	√
Black	√	UV-Vis-IR	√	√
Transparent clear	√	-	-	√
Transparent yellow	√	UV	-	√

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