IMAPlate™ 5RC96 Application Note

A simple solution
to improve the detection sensitivity of ELISA

Introduction

ELISA is an easy, specific, sensitive and economic biomolecule quantification method and is used by scientists as one of the first selected tools for viruses, bacteria, proteins and small molecules quantification because a diverse range of reagents is commercially available. But the detection sensitivity of the ELISA may likely be insufficient using a standard protocol for the measurement of biomolecules with low abundance or of some requiring high dilution in order to eliminate the matrix effect because the total amount of biomolecule may be under the detection limits of the conventional ELISA setup.

IMAPlate™ 5RC96 is the world's first miniaturized analytical platform capable of manually performing high-throughput liquid transfer, analysis, reaction and assay. It comprises 96 identical, funnel-like reaction units, positioned according to the standard 96-well plate format and each reaction unit contains a 5 µl round reaction chamber that can be used as a micro-cuvette being measurable by a plate reader. The reaction chamber can reach a light-path of 5mm with only 5 µl of solution, whereas the 96-well ELISA plate with flat bottom needs about 175 µl to reach 5mm height. Therefore, using IMAPlate™ 5RC96 for the readout of ELISA with100 µl reaction volume can simply increase the absorbance value approximately 1.7-fold.

ELISA usually uses the same 96-well ELISA plate for the reaction and the readout. The volume of reagents, sample and substrate solution used in the reaction is nearly the same and thus the conventional ELISA setup actually has no molecular enrichment although the molecule immobilization in ELISA is very similar to affinity chromatography. Since the IMAPlate™ 5RC96 can use only 5 µl of solution for the measurement, using IMAPlate™ 5RC96 for the readout of ELISA can dramatically reduce the substrate solution for the reaction. Therefore, the concentration of immobilized enzymes increases correspondently in the reaction with the reduction of the substrate solution and in turn, the concentration of colored product will be higher as well. For example, when using 25 µl of the substrate solution instead of 100 µl in the reaction, the concentration of the colored product is about 4 times higher if all immobilized enzymes are able to react with the substrate. Combined with the synergy of the longer light path of the IMAPlate™ 5RC96 the described ELISA setup can therefore increase absorbance values up to 7-folds.

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Experimental

Reagents and Materials

- Ready to use ELISA kit or ELISA reagent kit
- 96-well ELISA plate
- Pipette
- IMAPlate™ 5RC96 start kit
- Microwell plate reader

*Procedure for ELISA using the IMAPlate™ 5RC96 for result readout**

1. Coat 96-well ELISA plate according to the protocol provided in the reagent kit with capture antibody. *A reduced volume for coating (e.g. 50 µl for flat bottom or 40 µl for V and U bottom) may reduce the background.*
2. Block remaining protein-binding sites of the well with blocking reagent.

Note: above preparation steps are only for ELISA reagent kit

3. Perform the ELISA according to the protocol provided by manufacturer. *In order to increase the detectable rate, an increased volume of sample may be preferred for low abundant molecule measurement.*
4. **Add reduced volume of substrate solution e.g. TMB solution and vortex for 15 to 30 minutes.** *The volume of substrate solution used depends on the demands of the sensitivity and the bottom format of the 96-well plate. 25 µl would be the low limit for a flat bottom plate, otherwise not all immobilized enzymes are able to make contact with the substrate solution. If the volume of the substrate solution is the same as that for coating, the vortex is not necessary.*
5. After incubation, transfer 5 µl of the substrate solution (with or without the addition of acidic stop solution) from 96-well ELISA plate to IMAPlate™ 5RC96 by pipette or aspirate by capillary force, if the volume is large enough (e.g. 75 µl for flat bottom or 20 µl for V and U bottom). *It is suggested to measure the substrate solution without addition of acidic stop solution because it may cause the precipitation for standards and samples with high concentration and make the absorbance value of these standards and samples invalid.*
6. Place the IMAPlate™ 5RC96 in the reader with the adaptor and measure absorbance at wavelength of 665 nm and base line absorbance at wavelength of 500 nm without acidic stop solution or at wavelength of 450 nm and base line absorbance at wavelength of 650 nm with acidic stop solution. *For standards and samples having absorbance value over the upper detection limit of the reader, pipetting 1 µl or 2 µl to IMAPlate™ 5RC96 for the measurement can make these standards and samples valid and may extend the upper detection limit of the ELISA.*
7. Use true absorbance values ($A_{665} – A_{500}$) or ($A_{450} – A_{650}$) to calculate the concentration of samples according to the standards.

**Patent application has been filed.*

Results and Discussion

As predicted, the detection sensitivity and the low detection limit can be significantly improved with the new ELISA setup. Figure 1 shows the plot of two ELISA data sets for human TNF-a standard curves with a series of 3-fold dilution. The red curve is the plot of the data from the conventional ELISA setup and the green one is the plot of the data from the new ELISA setup. Both ELISAs were performed in 96-well plate with flat bottom according to the protocol provided in the kit except that, for the green curve, 25 µl of TMB substrate solution was used instead of 100 µl for the incubation with vortexing and the result was read out using the IMAPlate™ 5RC96 with the volume of 5 µl. The detection sensitivity (the slope of the initial increase) was increased about 7-fold and the low detection limit also went down correspondently. If a 96-well plate with V or U bottom is used and the substrate solution is reduced to 15 µl, the detection sensitivity are expected to increase more than 10-fold and the low detection limit can go down 10-fold as well. The detectable rate for those low abundant analytes can be increased further when an increased sample volume is applied. It has to be pointed out that to use higher concentration substrate solution would be preferred in order to avoid quickly running out of the substrate for standards of high concentration defined in the provided protocol.

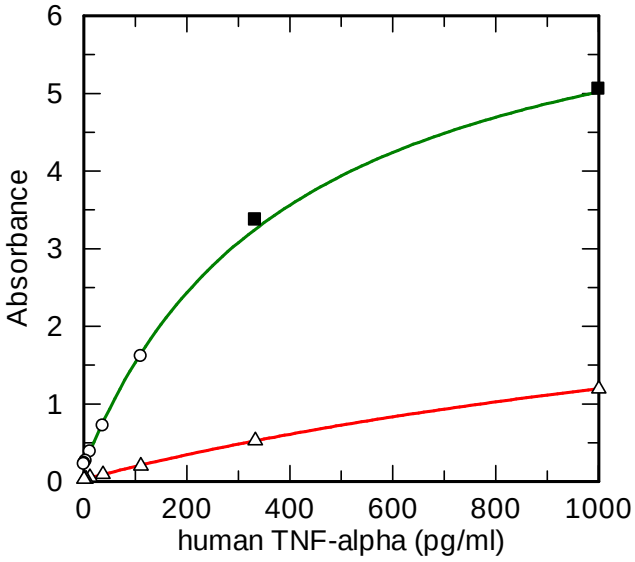

Figure 1

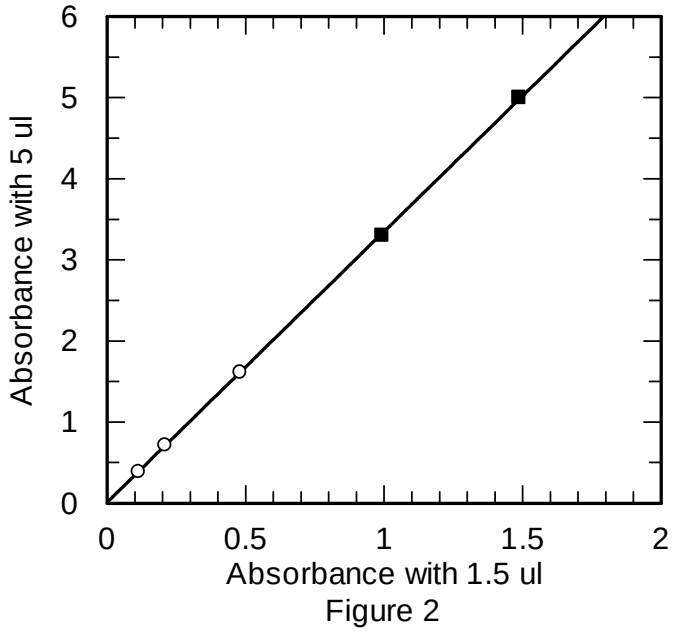

Figure 2

Since the absorbance value of two standards with the highest concentration was over the upper detection limit of the plate reader, a second measurement using 1.5 µl of the reaction solution was performed. Because the obtained absorbance value with 1.5 µl of the reaction solution decreased about 3.3-fold and matched the calculation based on the decrease of the light path, the linear fitting curve of standards in Figure 2 is used to derive the absorbance data for the two standards with 5 µl. Alternatively, it is also practical to measure the absorbance value directly from the 96-well ELISA plate after addition of 100 µl of suitable buffer and derive the data from the linear fitting curve of the standards in both measurements.

Conclusion

Using the IMAPlate™ 5RC96 for the ELISA readout can dramatically increase the detection sensitivity of commercial ELISA kit with the described ELISA setup, in which the ELISA is basically carried out according to user's protocol, except the readout step. So scientists can directly use commercial ELISA kits and perform the ELISA with their familiar routine procedure but the addition of several lower standards and the alternation of the readout. The described approach provides scientists a very simple but very effective method to improve the detection sensitivity of ELISA and will accelerate the discovery and applications of low abundant proteins in research and diagnostics.

The IMAPlate™ 5RC96 is an easy-to-use, robust, miniaturized analytical platform and the multi-utility of IMAPlate™ 5RC96 and the approach described will have a substantial impact not only on ELISA, but also on other type of assays.

Products 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	IMAPlate™ 5RC96 (White)	5 plates / box
NCL-P5B-004	IMAPlate™ 5RC96 (Black)	5 plates / box
NCL-P5T-006	IMAPlate™ 5RC96 (Clear)	5 plates / box