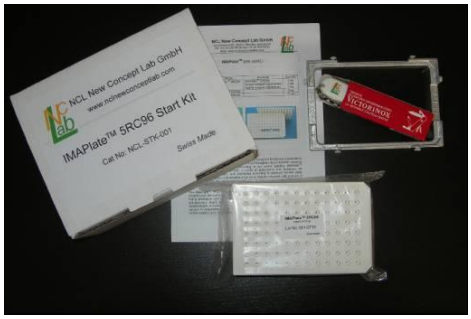


IMAPlate™ 5RC96 Application Note

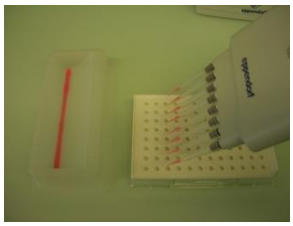
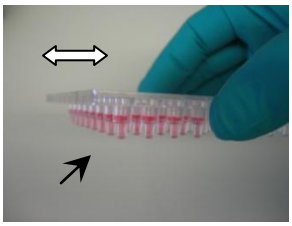
Miniature, high sensitive ELISA with Macro Volume (15-25 µl)

Introduction

The enzyme linked immunosorbent assay (ELISA) is fundamental immunological and biochemical technique and is routinely used to evaluate the presence of particular antigens or antibodies in a specimen. It is usually performed in a 96-well plate. Though attempting to miniature the assays in 384-well plate has been shown possible, it encounters problems such as the formation of air gap the reaction wells, the relative large dead volume and difficult for handling either manually or by automation. The existence of air gap at the bottom or on the side wall can prevent the assay solution to access to the well surface and cause incomplete antigen and antibody reaction. The relative large dead volume can markedly reduce the washing efficiency and result in high background.

IMAPlate™ 5RC96 comprises 96 identical, funnel-like bottomless reaction units. Using IMAPlate™ 5RC96 to perform miniature ELISA can overcome many obstacles observed in the process of miniaturization of ELISA to 384- and 1536-well plate format. The unique characteristics of the IMAPlate not only provide air-gap-free pipetting, but also enable the assay solution to be discarded completely from the reaction units. The simple "go-through washing procedure" provides a highly efficient way to remove the unreacted reagent and eliminates cross contamination between each sample as well. The IMAPlate macro volume miniature ELISA can fit for both manual operation and automated workstation.

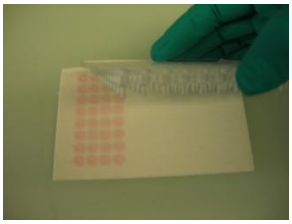
IMAPlate macro volume miniature ELISA general procedure

I. Coating

1. Place a new IMAPlate on an empty F-bottomed 96-well plate and pipette 20 µl of capture antibody into the upper compartment of each reaction unit.
2. Gently tap the IMAPlate in horizontal direction to ensure the solution filling up the lower compartment. Incubate 30 minutes at room temperature after covering the IMAPlate with a wet filter paper or closing in a plastic zip bag.

II. Washing



3. Empty the IMAPlate by placing it on a thick filter paper and pushing slightly against the filter paper for several seconds. After the solution is completely absorbed by the filter paper, put it back on the empty F-bottomed 96-well plate.
4. Wash the IMAPlate three times with the "go-through washing procedure" -- pipette 50 µl of wash buffer into the upper compartment and empty it with a thick filter paper.
5. After the last wash, invert the plate and gently strike it against clean paper towels to remove any remaining wash buffer. Put it back on the empty F-bottomed 96-well plate.

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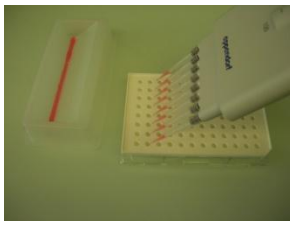
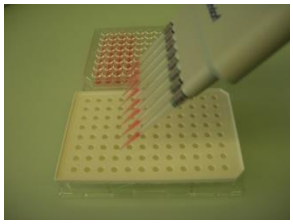


III. Blocking

6. Pipette 40 µl of blocking solution into the upper compartment.
7. Gently tap the IMAPlate in horizontal direction to ensure the solution filling up the lower compartment. Cover the IMAPlate with a wet filter paper and incubate 10 minutes at room temperature.

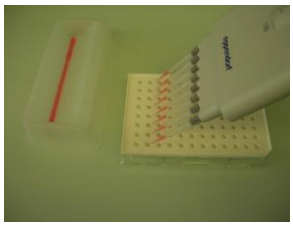
IV. Washing (repeat step 3 to 5)



V. Standards and sample loading

8. Pipette 20 µl of standards and samples into the upper compartment.
9. Gently tap the IMAPlate in horizontal direction to ensure the solution filling up the lower compartment. Cover the IMAPlate with a wet filter paper and incubate 30 minutes at room temperature.

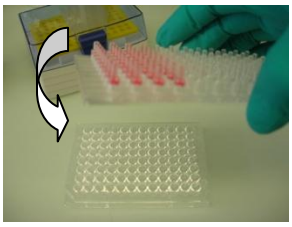
VI. Washing (repeat step 3 to 5)



VII. Enzyme conjugate loading

10. Pipette 20 µl of capture antibody into the upper compartment.
11. Gently tap the IMAPlate in horizontal direction to ensure the solution filling up the lower compartment. Cover the IMAPlate with a wet filter paper and incubate 30 minutes at room temperature.

VIII. Washing (repeat step 3 to 5)



IX. Colorimetric reaction

12. Pipette 20 µl of substrate solution into the upper compartment.
13. Gently tap the IMAPlate in horizontal direction to ensure the solution filling up the lower compartment and incubate 5 to 10 minutes at room temperature.
14. Stop the reaction by pipetting 5 µl of stop solution and mix by inverting the IMAPlate several times.

X. Reading IMAPlate

15. Place the IMAPlate into an IMAPlate adaptor and load them to a 96-well plate reader. Measure absorbance at both peak and base-line wavelength
16. Calculate the true absorbance ($Abs_{true} = Abs_{peak} - Abs_{baseline}$) and use the true absorbance for data analysis.

Example

The figure on the right presents the standard concentration curves of human transferring ELISA. The line with solid circle was resulted from IMAPlate with a reaction volume of 20 µl and the line with open circle from Nunc Maxisorp F-96-well plate with a reaction volume of 100 µl. **Both ELISA were performed with the same condition: same reagent concentration and same incubation times.**