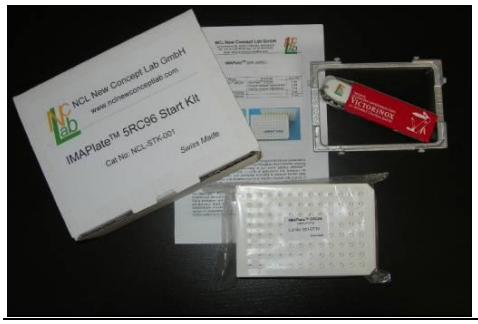


IMAPlate™ 5RC96 Application Note

Miniature, high sensitive homogeneous assays

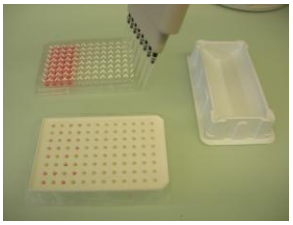
Introduction

Due to relatively low cost of absorbance plate readers, cheap and uncomplicated reagents preparation, low sample consumption and simple "add-mix-measure" procedure, 96-well plate based homogeneous colorimetric assays are still popularly used in the labs for routine analysis, research application and compound screening. Attempting to miniature the assays in 384- or 1536-well plate seems not practicable for manual pipetting, and remains a challenge even for automations. Although the "add-mix-measure" assay procedure is straightforward, false results can easily be generated due to the air gap between the solutions caused by multiple pipetting into a small well.

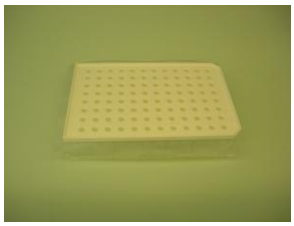
IMAPlate™ 5RC96 comprises 96 identical, funnel-like bottomless reaction units, in which the diameter of the upper compartment is larger than the well of 384-well plate while the diameter of the lower compartment is smaller than the well of 1536-well plate. The unique design of the IMAPlate enables each reaction unit to hold more than 50 µl of solution. When the lower bottom opening is sealed, the solution pipetted into the upper compartment does not flow into the lower compartment. For example, by using a parafilm sheet to temperately seal the bottom, reagent and sample can stay in the upper compartment for thoroughly mixing and incubation. Once the parafilm sheet removed, assay solutions will flow and fill up the lower compartment, and immediately increase total liquid thickness about 5 mm. Therefore, for the absorbance measurement, the light path length is increased ultimately. Using IMAPlate™ 5RC96 can overcome the incomplete mix and reaction observed in 384- and 1536-well plate based assays. It not only reduces the assay volume, but also keeps high or increases the sensitivity of the assay. The IMAPlate™ 5RC96 based miniature homogeneous assay can fit for both manual operation and automated liquid handling workstation.

IMAPlate Macro Volume Miniature Assay Procedure

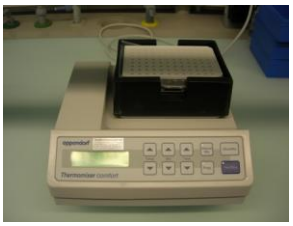
1. Prepare a piece of parafilm (e.g. 100 x 150 mm) and place it on the top of an empty 96-well plate without the wax paper.



3. Add assay components one by one into the upper compartment. (*Total assay volume* is recommended between 15 µl to 25 µl.*)



2. Push a new IMAPlate down to the 96-well plate and the parafilm will automatically seal the bottom openings.

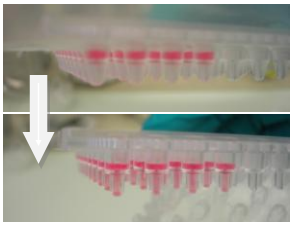


4. Mix thoroughly with a plate shaker and incubate.

NCL New Concept Lab GmbH
www.nclnewconceptlab.com

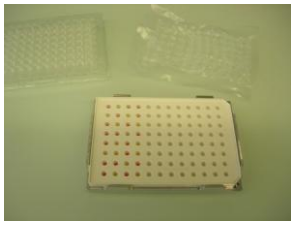
Eichenstrasse 22
CH 4313 Moehlin
Switzerland

Tel: +41 61 853 08 20
Fax: +41 61 853 08 23
e-mail: info@nclnewconceptlab.com

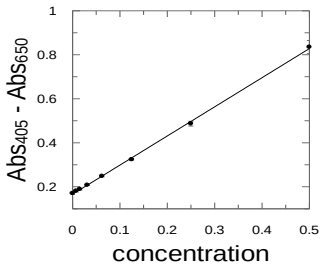


5. Before measurement, peel off the parafilm and the assay solution will flow into the lower compartment.

7. Load the IMAPlate adaptor with the assay IMAPlate into the reader and measure both the peak absorbance and base line absorbance.



6. Place the IMAPlate in an IMAPlate adaptor and gently tap it in horizontal direction several times to ensure the assay solution filling up the lower compartment.



8. Use true absorbance values ($Abs_{peak} - Abs_{base-line}$) to plot the standard curve and calculate the concentration of samples.

* *The assay volume should not be over the maximum volume, which the IMAPlate can hold to withstand each step of the assay procedure from dropping off.*

Examples

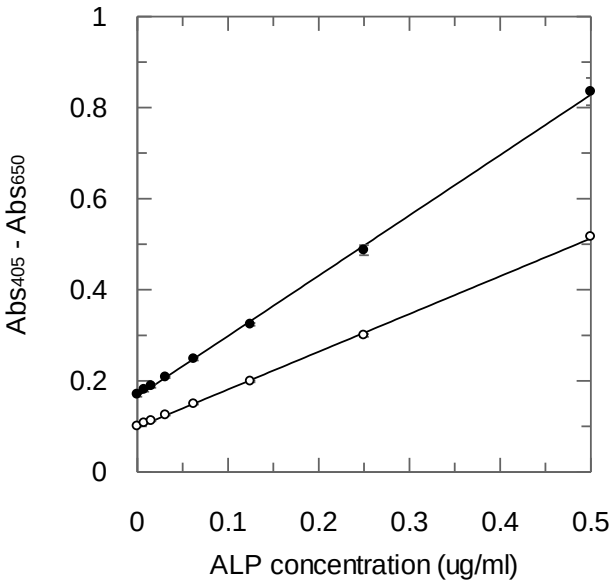


Figure 1. Concentration curves of Alkaline Phosphatase: the line with solid circle is performed **in IMAPlate with total 20 µl** of assay solution and the line with open circle is performed **in Nunc 96-well plate with 150 µl** of total assay solution.

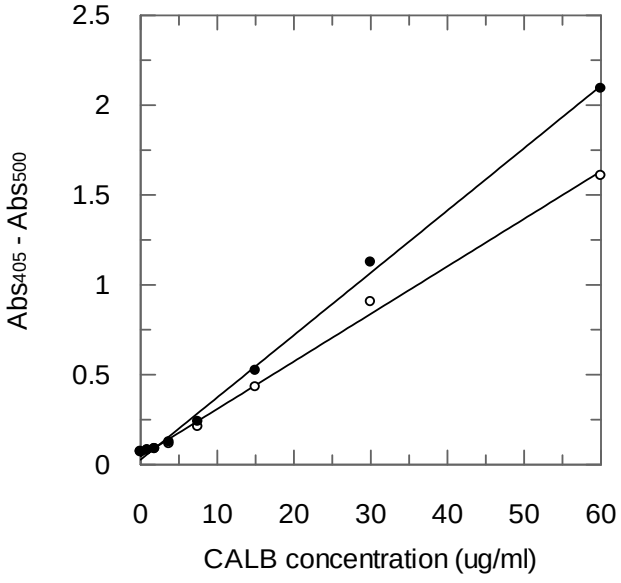


Figure 2. Concentration curves of Candida Anterctica Lipase B: the line with solid circle is performed **in IMAPlate with total 20 µl** of assay solution and the line with open circle is performed **in Nunc 96-well plate with 200 µl** of total assay solution.