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Using IMAPlate 5RC96 to enhance the sensitivity of Human Cytokine ELISA MAX™ Deluxe from BioLegend

INTRODUCTION: IMAPlate 5RC96 is a miniature multi-usage 96-well formatted lab device. It can be used as micro volume long path-length cuvette array to measure up to 96 samples at a time. The use of IMAPlate 5RC96 to enhance the sensitivity of conventional ELISA is based on the observation that the absorbance of the final reaction mixture of ELISA in the 96-well plate mainly depends on the amount of the bound enzyme in the well, but not on the amount of substrate solution because of the inverse linear relationship between the path-length of the well and the concentration of the color solution. However, if the mixture is transferred to a fixed path-length cuvette for measurement, less substrate solution can generate higher concentration of color solution and in turn it will get higher absorbance. Therefore, by reducing both substrate solution and stop solution and transferring the reaction mixture to the long path-length, low volume IMAPlate 5RC96 for measurement, it can markedly increase the slope of absorbance of the final reaction mixture vs concentration of the analyte - the sensitivity of ELISA.

ASSAY PROCEDURE: (*follow the same procedure provided in the kit except the decrease of volume in some steps.*)

Coat U-bottomed 96-well plate with **50µl*** of capture antibody in coating solution over night at 4°C

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Wash the coated plate with wash buffer 4 times following the instruction provided in the kit

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Add 100µl standard control and sample to each well; Incubate 120 minutes at RT with shaking

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Wash 4 times

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Add **50µl*** detection antibody solution to each well; Incubate 60 minutes at RT with shaking

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Wash 4 times

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Add **50µl*** Streptavidin-HRP conjugate to each well; Incubate 30 minutes at RT with shaking

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Wash 6 times

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Add 25µl* TMB solution; Incubate 15 minutes with 800 rpm shaking

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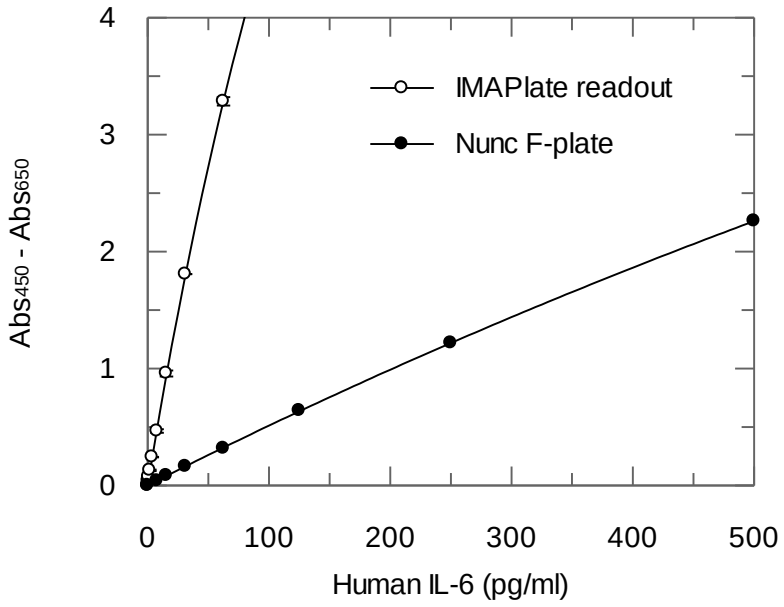
Add 5µl* stop solution and mix thoroughly; Transfer 25µl mixtures to IMAPlate and read at 450 nm with correction at 650nm.

*Note: the volume with * is reduced to ½ or more of required volume in kit procedure.*

RESULTS:

1) Comparison of IMAPlate readout of Human IL-6 ELISA performed in U-bottomed plate and Nunc F-plate readout performed in F-bottomed plate:



2) Comparison of IMAPlate readout of Human IL-17A ELISA performed in U-bottomed plate and Nunc. F-plate readout performed in F-bottomed plate: