

**Using IMAPlate 5RC96 to enhance the sensitivity of LEGEND MAX™ ELISA kits with pre-coated plates 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 light 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 till to the step of adding substrate solution)

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



Add 50µl assay buffer to each well



Add 50µl standard control and sample to each well; Incubate 2 hours at RT with shaking



Wash 4 times



Add 100µl detection antibody solution to each well; Incubate 1 hour at RT with shaking



Wash 4 times



Add 100µl Streptavidin-HRP conjugate to each well; Incubate 30 minutes at RT with shaking



Wash 5 times

**Kit plate readout**



Add 100µl TMB solution; Incubate 15 minutes



Add 100µl 2N H<sub>2</sub>SO<sub>4</sub> stop solution; Read Abs at 450nm with correction at 650nm.

**IMAPlate readout**



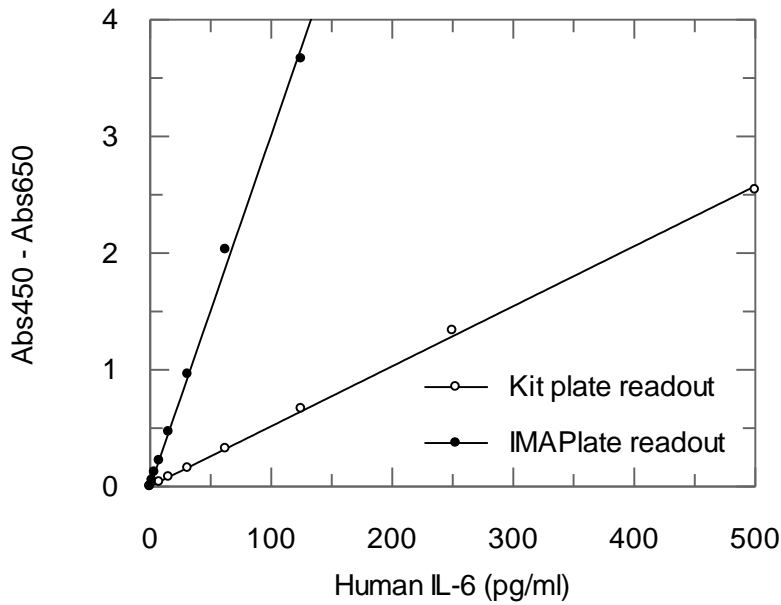
Add 40µl TMB solution; Incubate 15 minutes with 800 rpm shaking



Add 10µl stop solution; Transfer 40µl mixtures to IMAPlate and read at 450 nm with correction at 650nm.

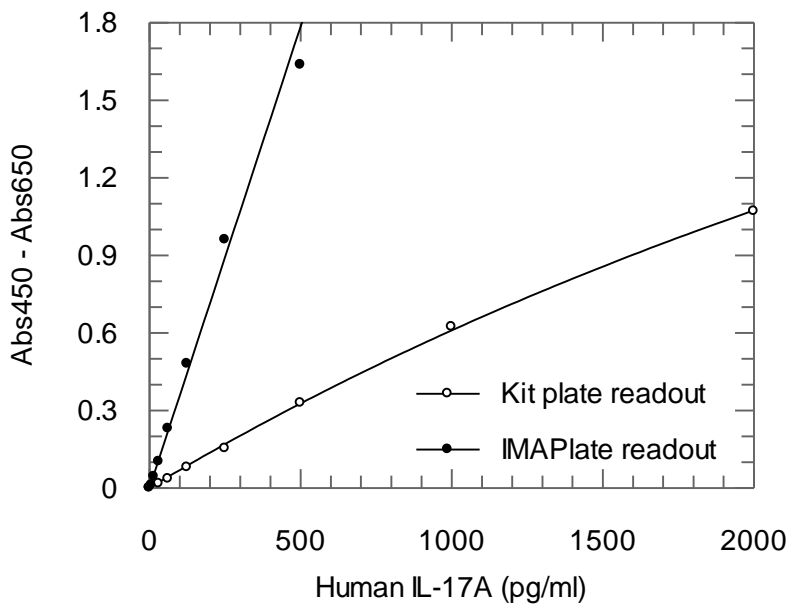
**RESULTS:**

1) LEGEND MAX™ Human IL-6 ELISA Kit:



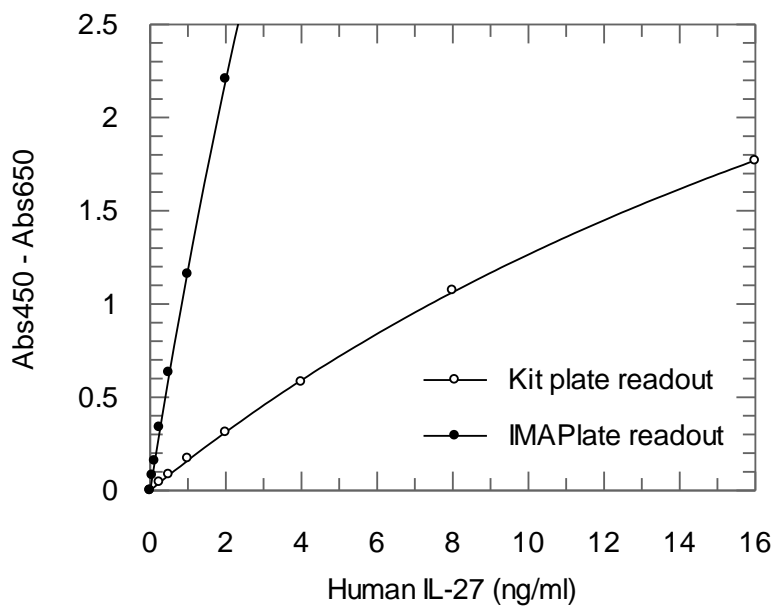
Kit plate readout (Abs450 - Abs650)			
pg/ml	Mean	SD	CV%
500	2.574	0.025	1.0
250	1.369	0.017	1.2
125	0.697	0.001	0.2
62.5	0.355	0.001	0.2
31.3	0.189	0.009	4.9
15.6	0.113	0.006	5.0
7.8	0.071	0.002	3.0
0	0.034	0.003	8.3
IMAPlate readout (Abs450 - Abs650)			
pg/ml	Mean	SD	CV%
125	3.822	0.052	1.4
62.5	2.186	0.127	5.8
31.3	1.118	0.046	4.1
15.6	0.624	0.007	1.1
7.8	0.378	0.004	1.1
3.9	0.279	0.016	5.6
1.95	0.208	0.010	4.8
0	0.159	0.001	0.4

2) LEGEND MAX™ Human IL-17A ELISA Kit:



Kit plate readout (Abs450 - Abs650)			
pg/ml	Mean	SD	CV%
2000	1.105	0.037	3.4
1000	0.657	0.016	2.5
500	0.363	0.016	4.3
250	0.187	0.008	4.5
125	0.114	0.007	6.2
62.5	0.070	0.002	3.1
31.3	0.051	0.001	1.4
0	0.035	0.000	0.0
IMAPlate readout (Abs450 - Abs650)			
pg/ml	Mean	SD	CV%
500	1.818	0.015	0.8
250	1.141	0.006	0.5
125	0.661	0.029	4.4
62.5	0.411	0.015	3.6
31.3	0.282	0.007	2.5
15.6	0.224	0.011	5.1
7.8	0.192	0.001	0.7
0	0.182	0.004	1.9

3) LEGEND MAX™ Human IL-27 ELISA Kit:



Kit plate readout (Abs450 - Abs650)			
ng/ml	Mean	SD	CV%
16	1.814	0.091	5.0
8	1.118	0.017	1.5
4	0.626	0.013	2.0
2	0.357	0.001	0.2
1	0.217	0.006	2.9
0.5	0.131	0.002	1.6
0.25	0.089	0.003	3.2
0	0.047	0.001	1.5
IMAPlate readout (Abs450 - Abs650)			
ng/ml	Mean	SD	CV%
4	overflow		
2	2.467	0.048	1.9
1	1.420	0.003	0.2
0.5	0.893	0.001	0.2
0.25	0.599	0.010	1.7
0.13	0.418	0.034	8.1
0.063	0.341	0.009	2.7
0	0.261	0.011	4.3