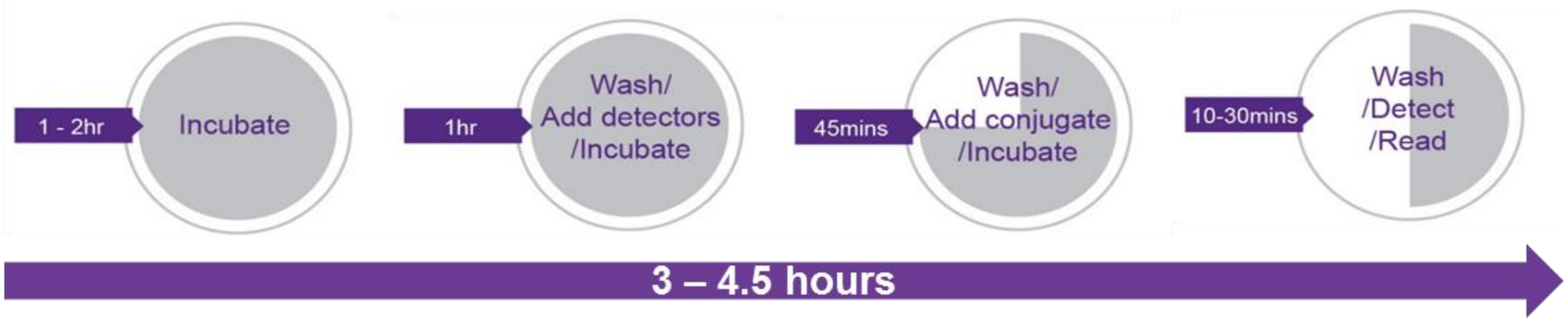
Calculating and evaluating ELISA data

Human PD-L1 ELISA Kit [28-8] (ab214565)

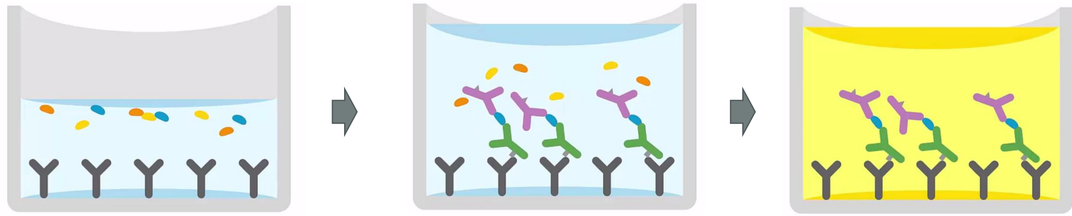
abcam

October 2016

Standard ELISA



One-step ELISA



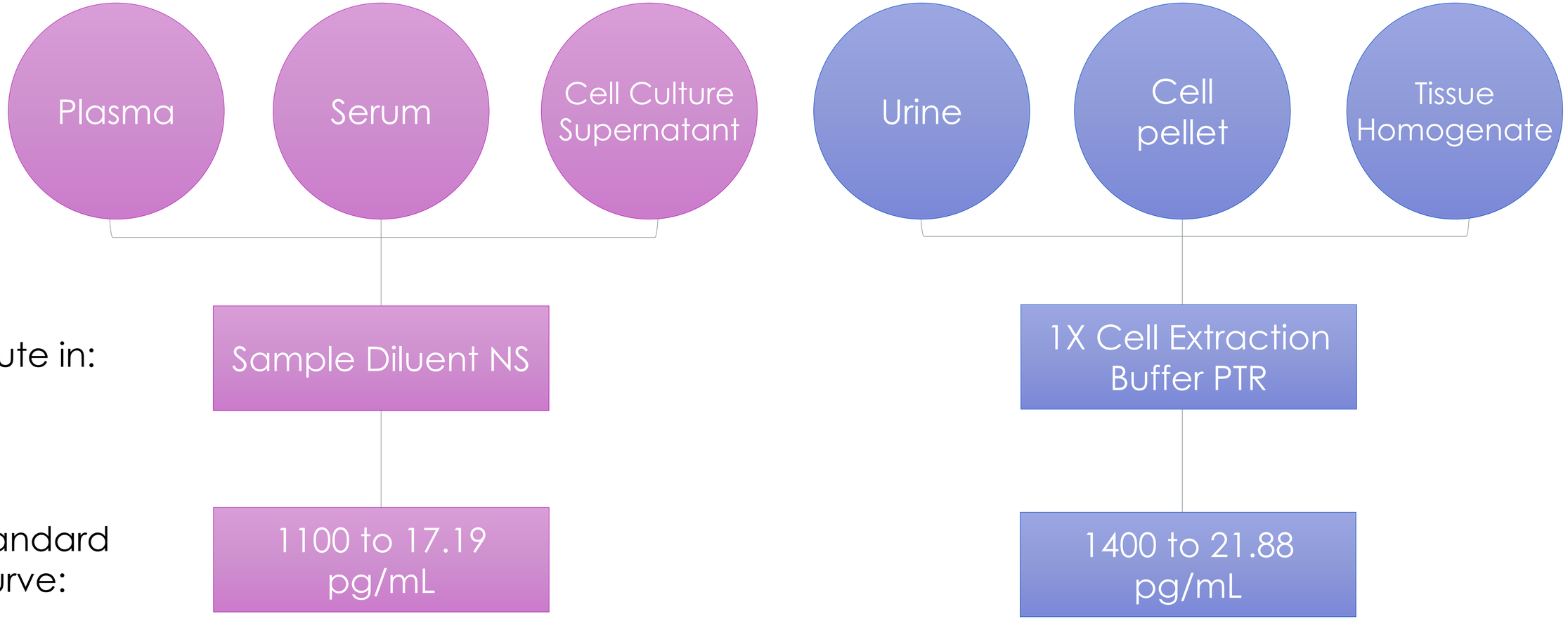
SimpleStep ELISA®

Human PD-L1 ELISA Kit [28-8] (ab214565)

Choosing the standard and preparing the plate

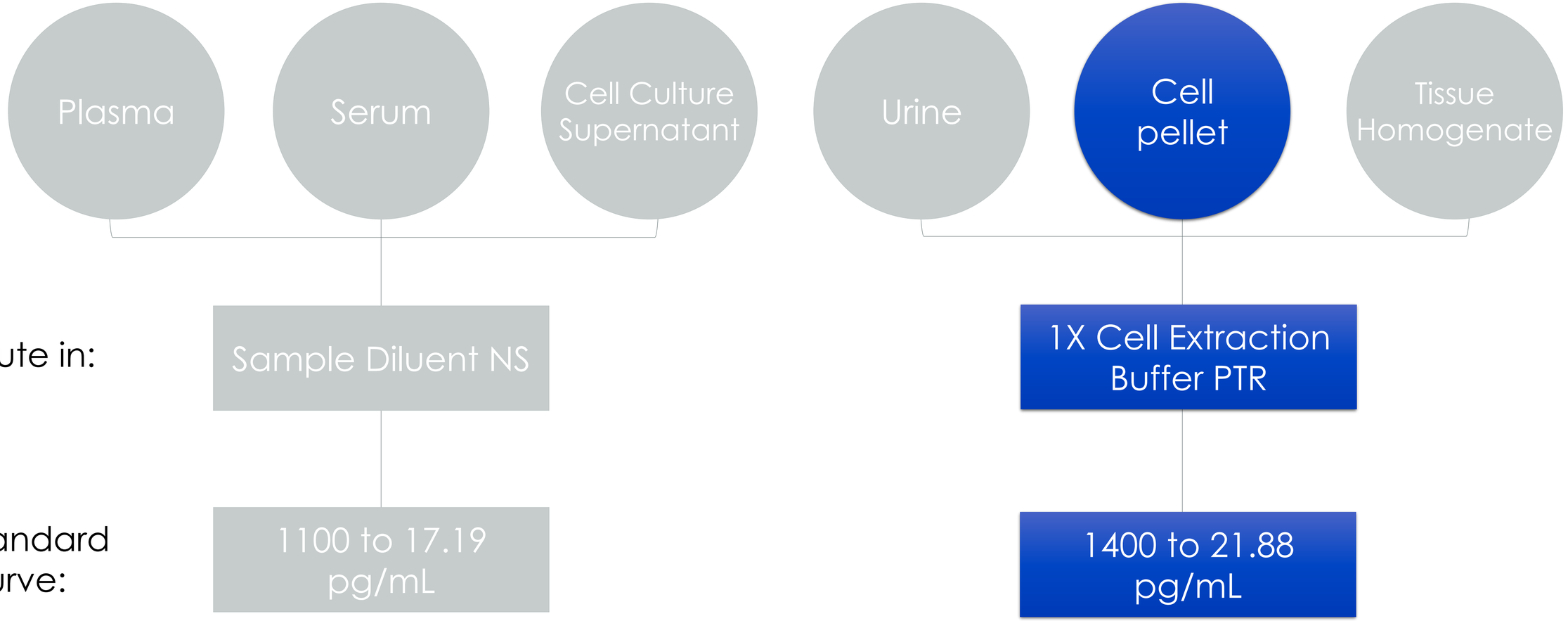
SimpleStep ELISA®

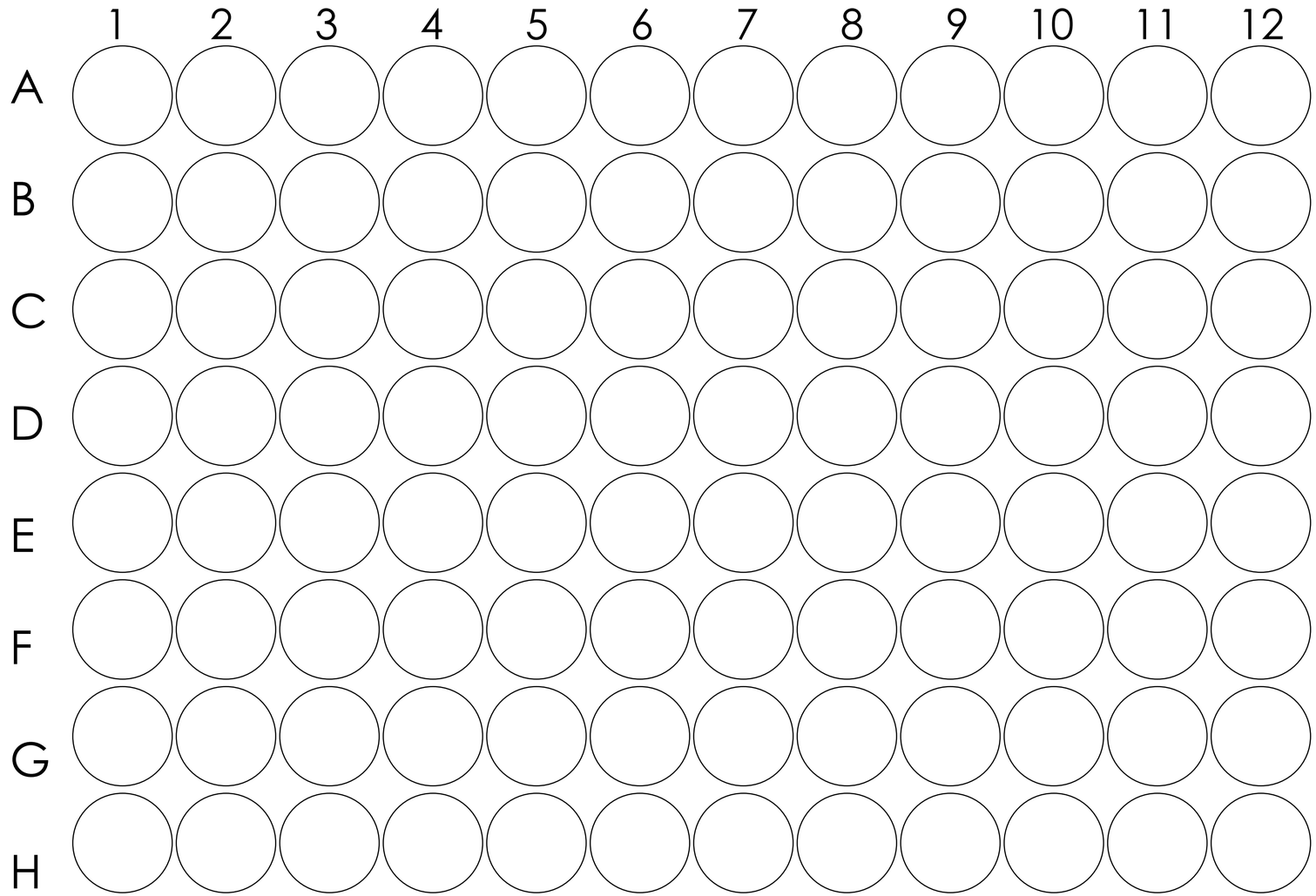
Human PD-L1 ELISA Kit [28-8] (ab214565)

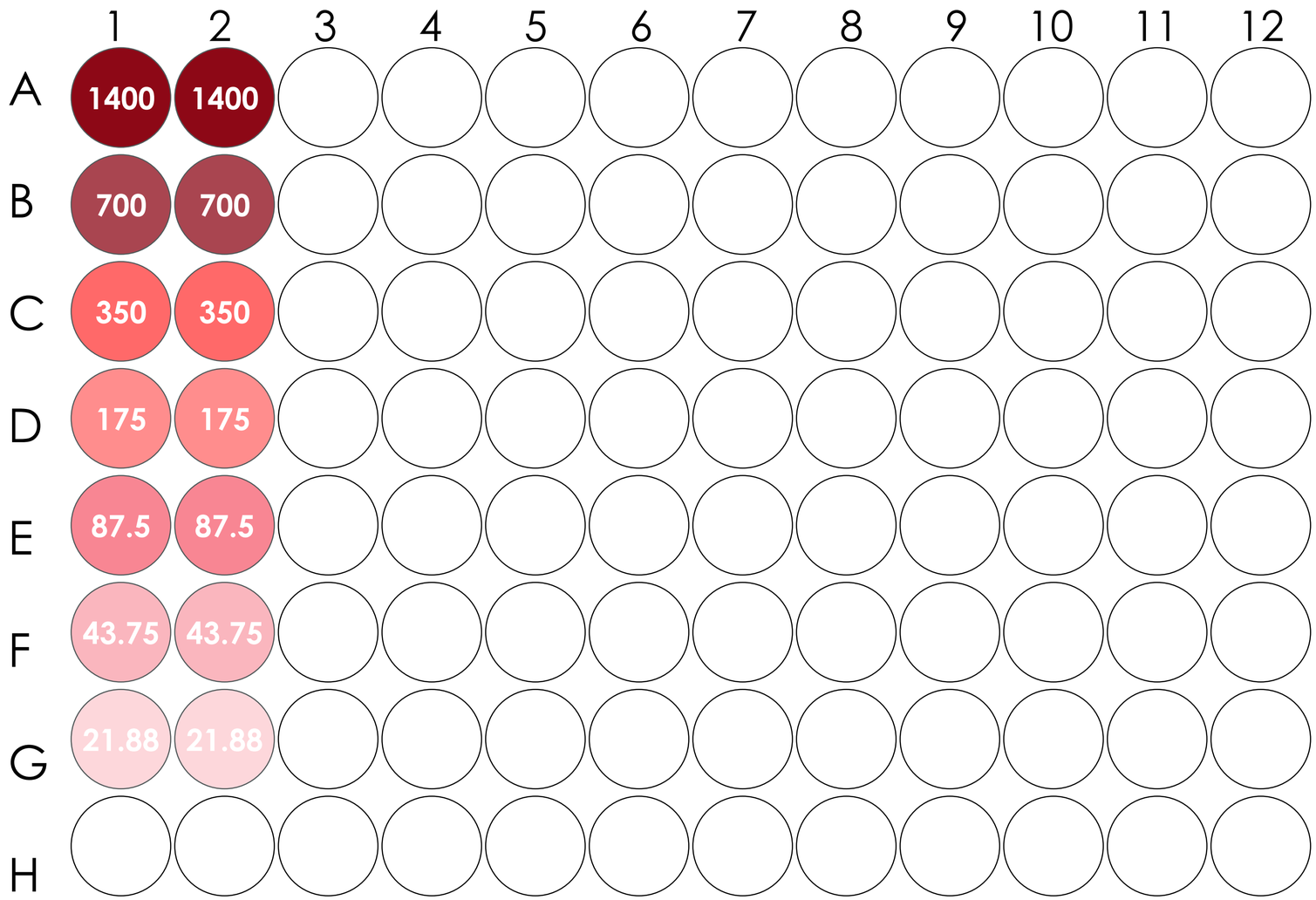


SimpleStep ELISA®

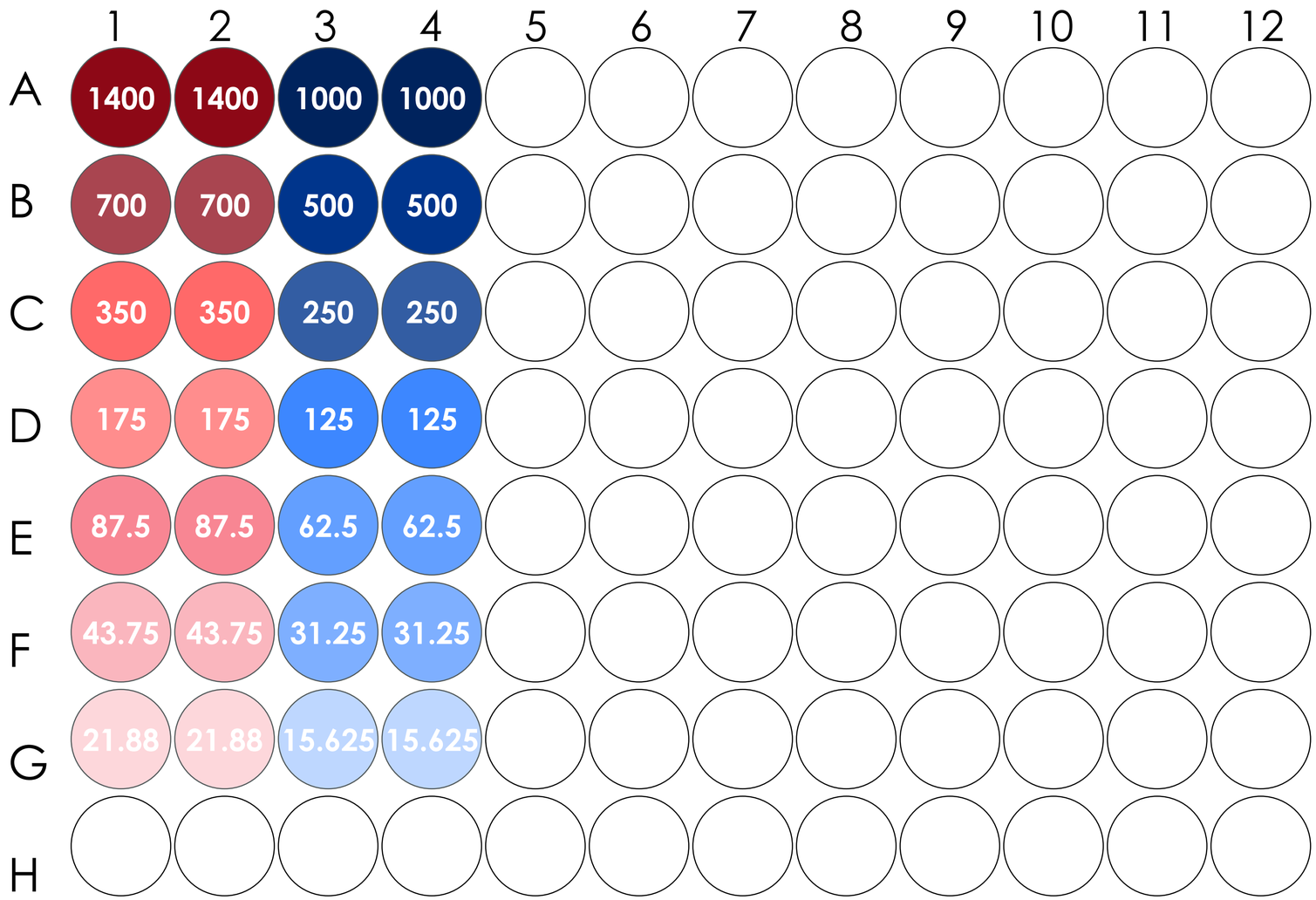
Human PD-L1 ELISA Kit [28-8] (ab214565)



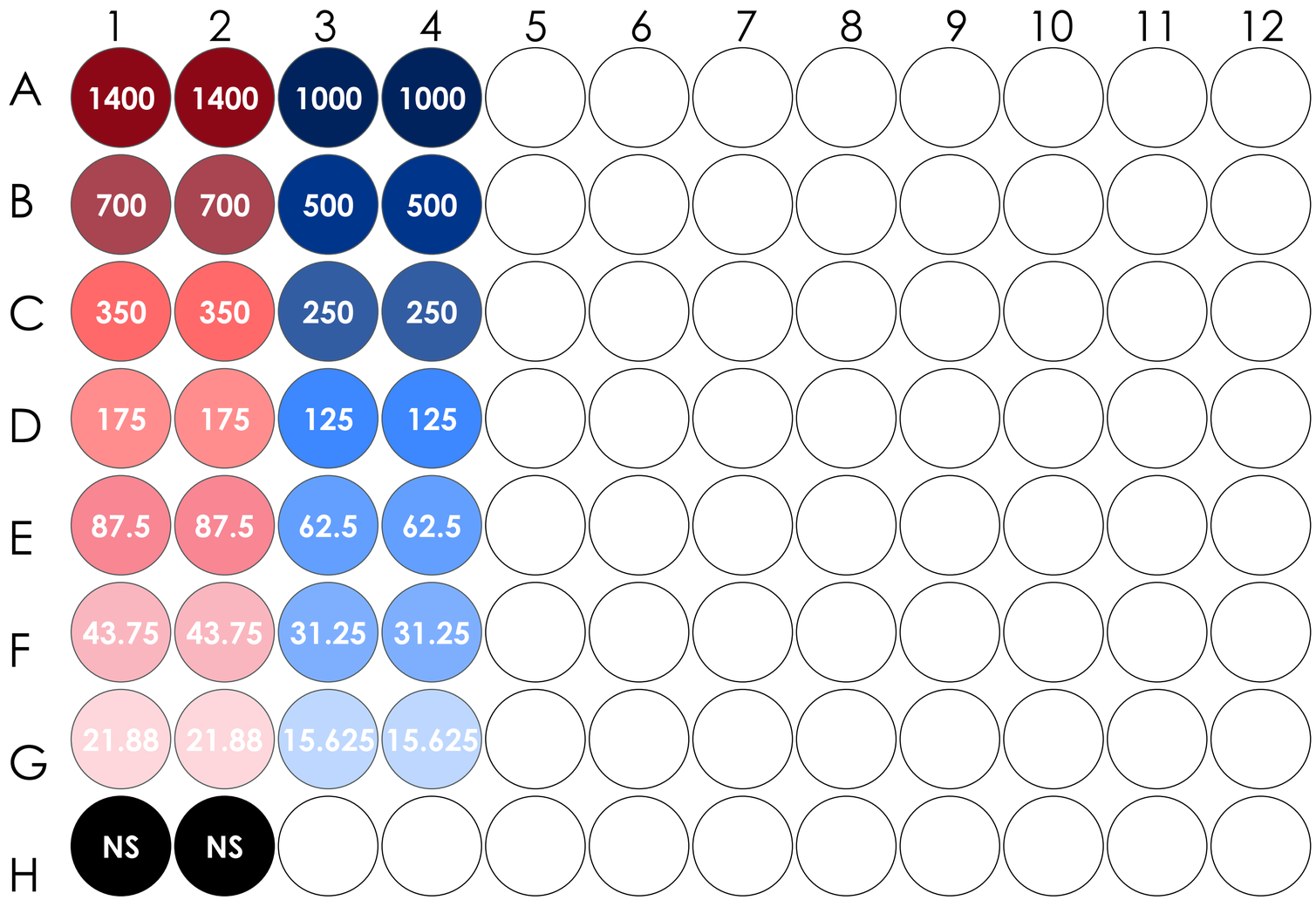

1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H



Standard
1,400 to 21.88 pg/mL

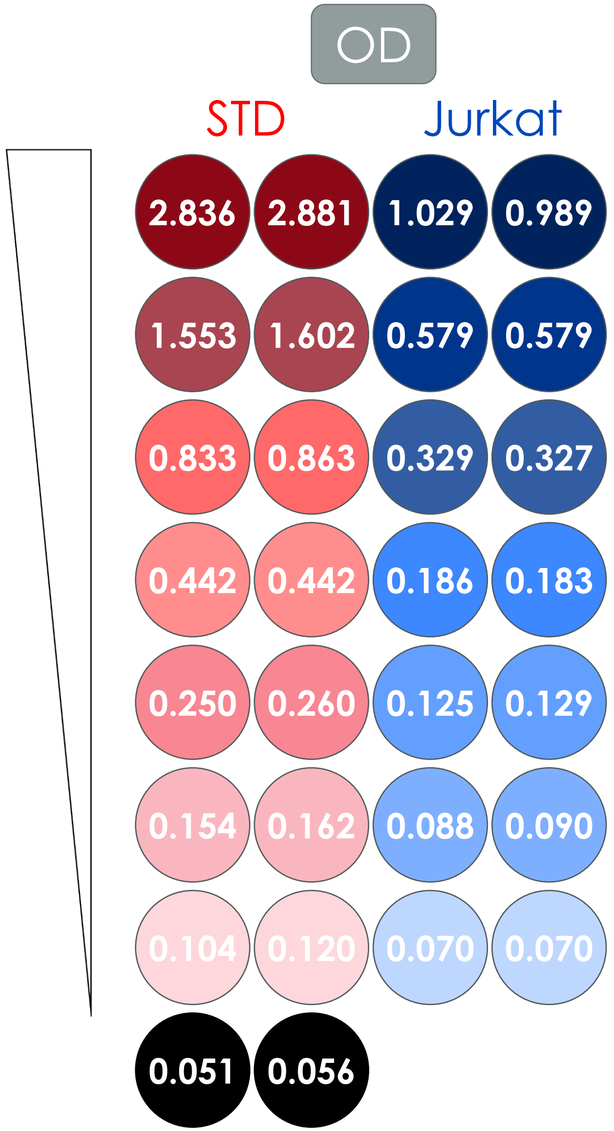


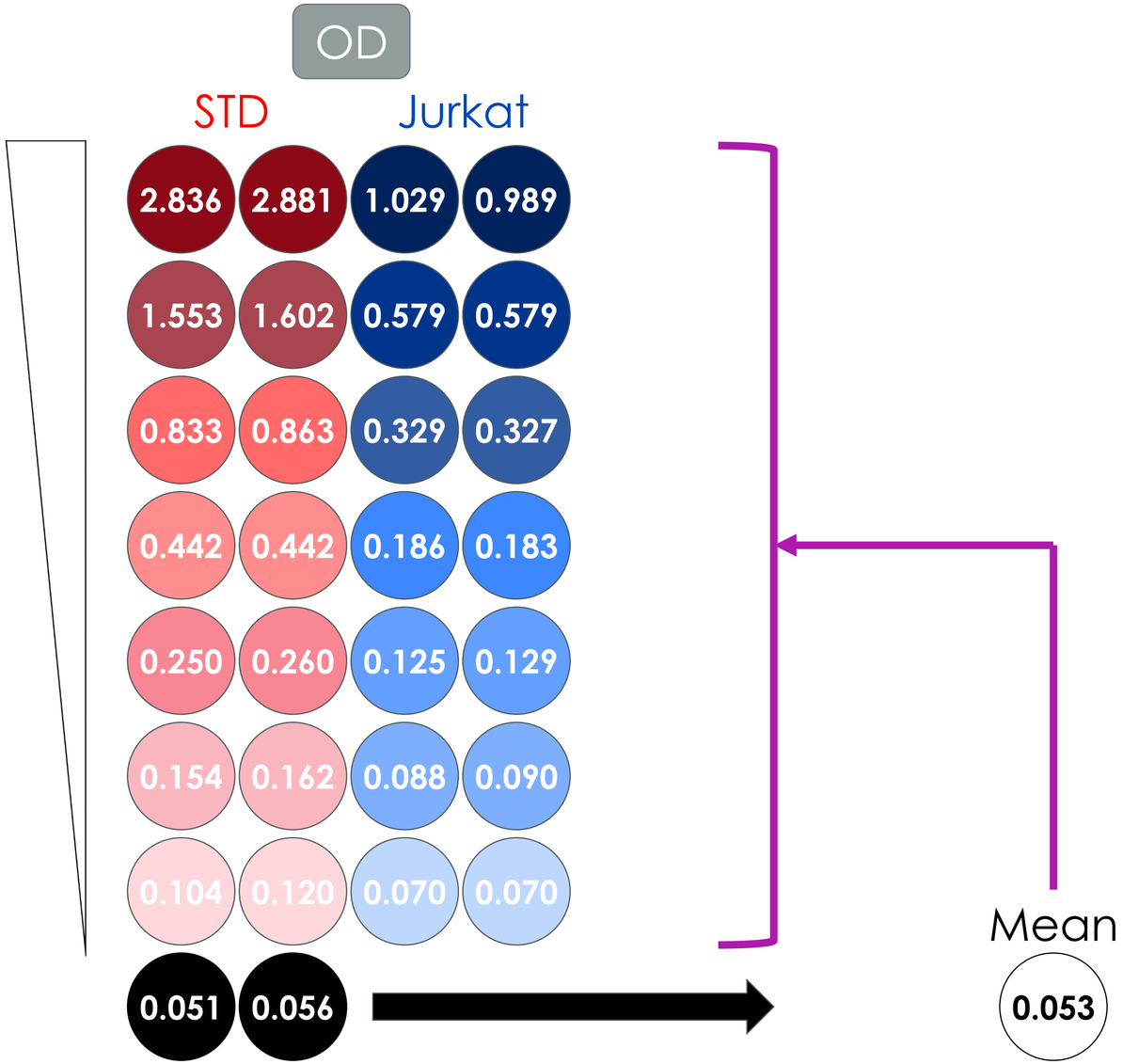
Standard
1,400 to 21.88 pg/mL
Jurkat+LPS+IFNg
1,000 to 15.625 µg/mL



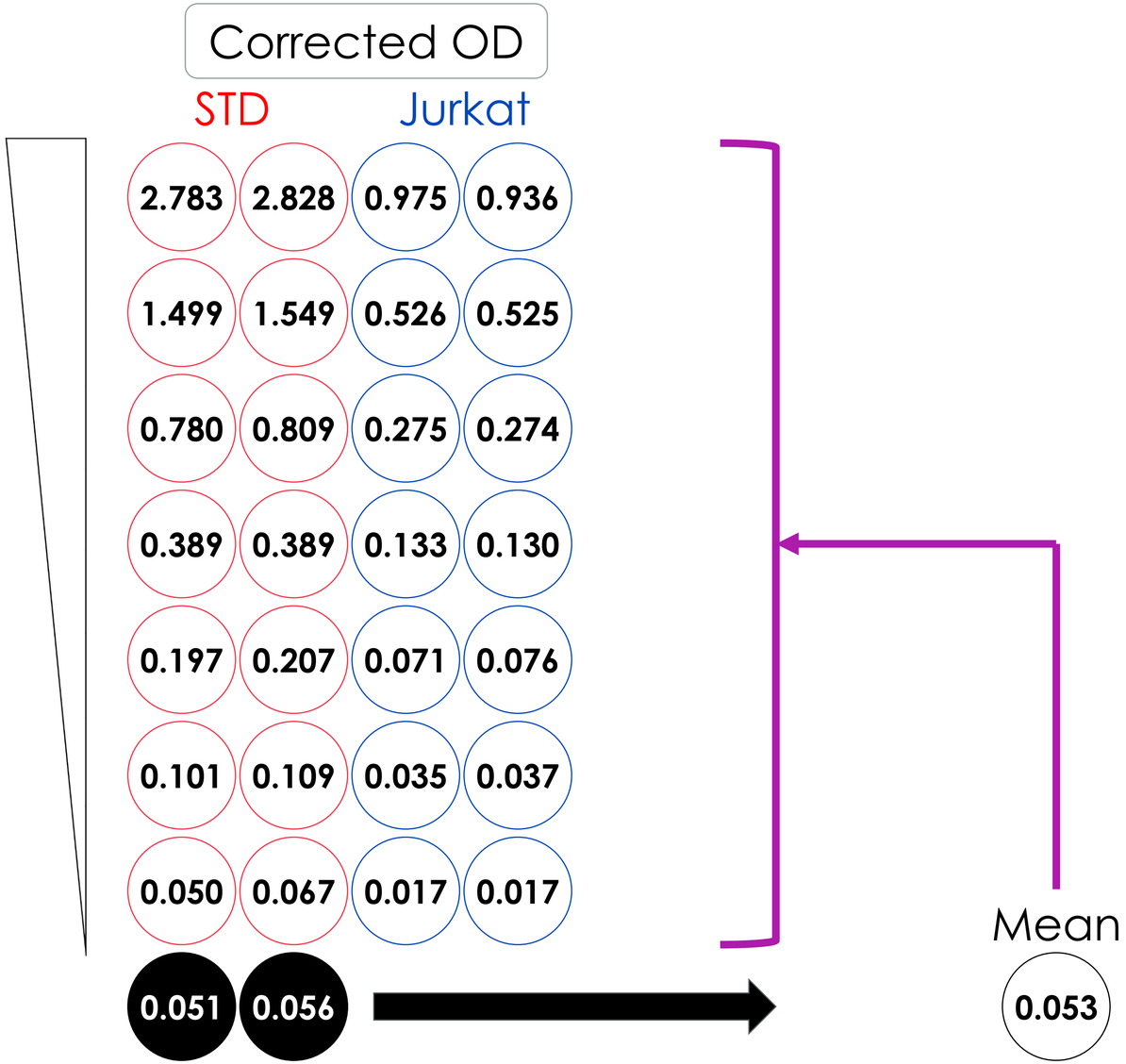
Standard
1,400 to 21.88 pg/mL
Jurkat+LPS+IFNg
1,000 to 15.625 µg/mL
Negative
Sample diluent

Verifying the validity of the measure


OD
STD
Jurkat
2.836
2.881
1.029
0.989
1.553
1.602
0.579
0.579
0.833
0.863
0.329
0.327
0.442
0.442
0.186
0.183
0.250
0.260
0.125
0.129
0.154
0.162
0.088
0.090
0.104
0.120
0.070
0.070
0.051
0.056



Subtract mean of the blank to all samples and standard points



Subtract mean of the blank to all samples and standard points

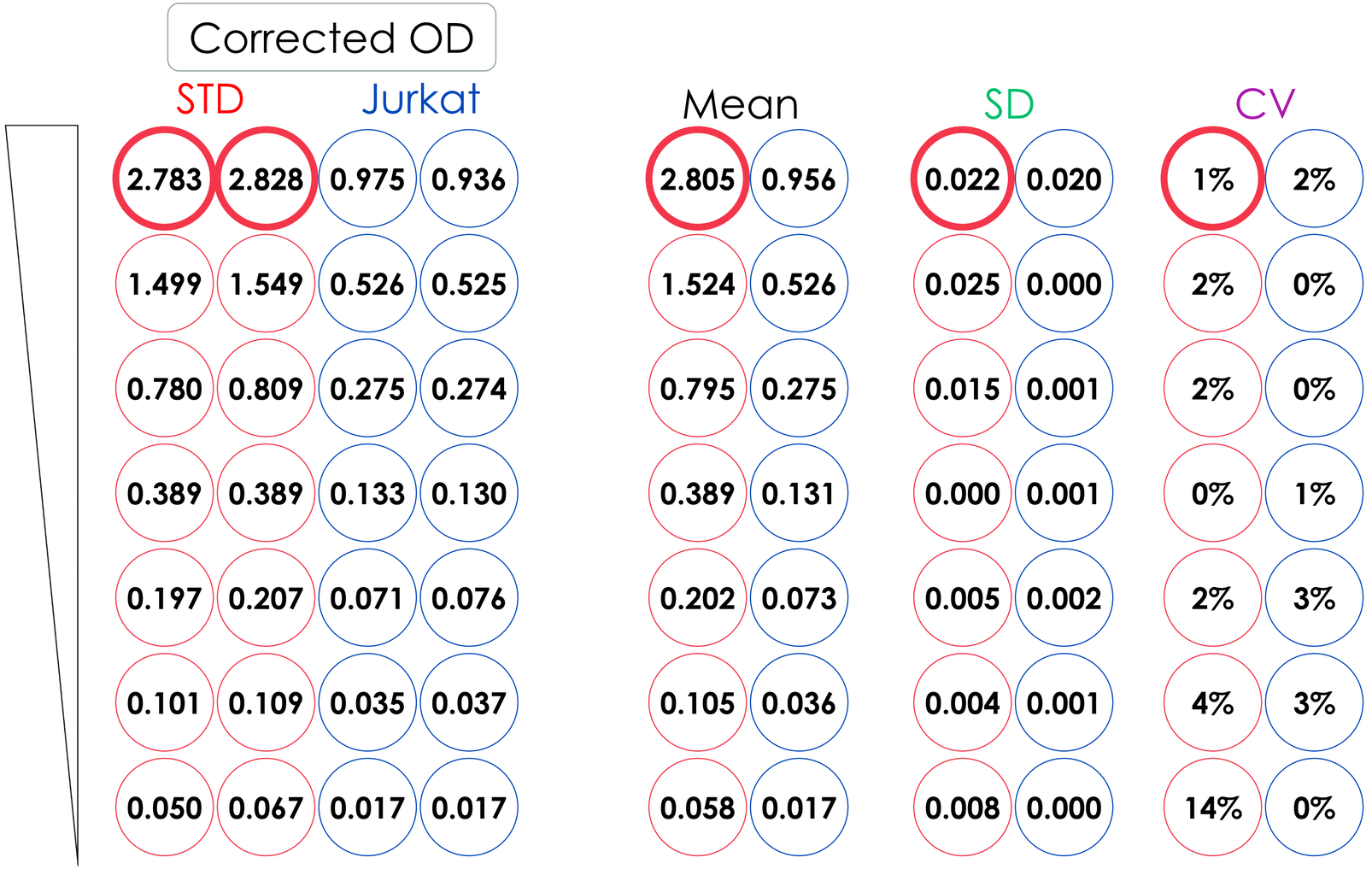
Corrected OD

STD		Jurkat	
2.783	2.828	0.975	0.936
1.499	1.549	0.526	0.525
0.780	0.809	0.275	0.274
0.389	0.389	0.133	0.130
0.197	0.207	0.071	0.076
0.101	0.109	0.035	0.037
0.050	0.067	0.017	0.017

CV: Coefficient of Variation

SD: Standard Deviation

$$CV = \frac{SD}{\text{Mean OD}} < 20\%$$

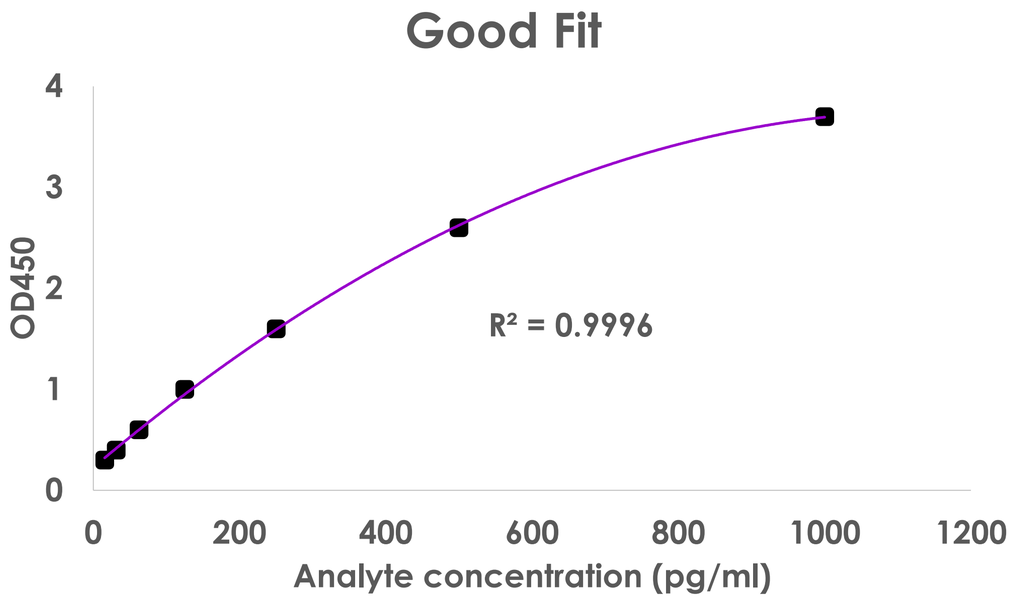


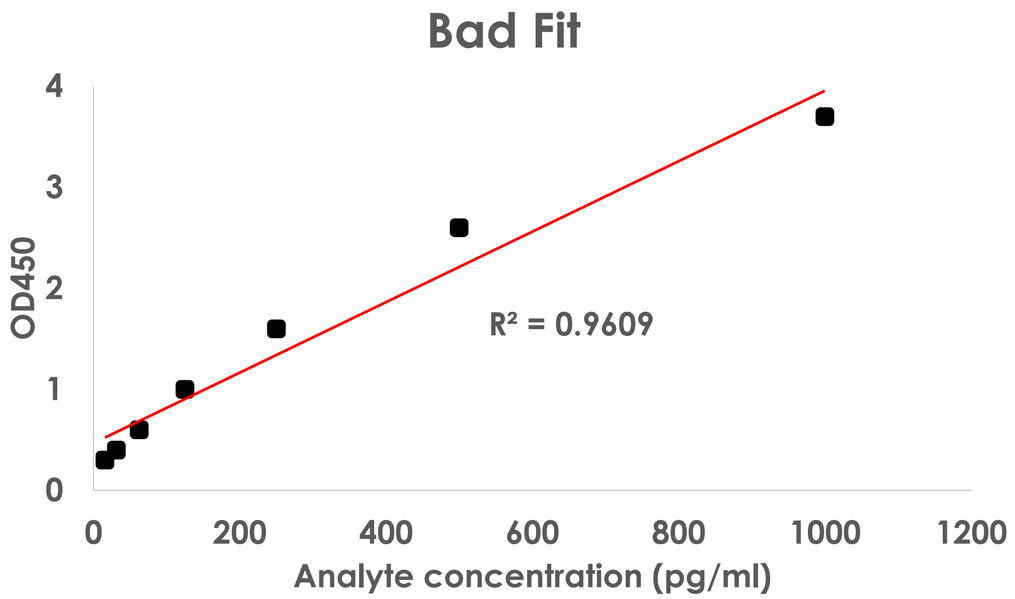
CV: Coefficient of Variation

SD: Standard Deviation

Creating the standard curve

Bioassay like ELISA have a sigmoidal curve



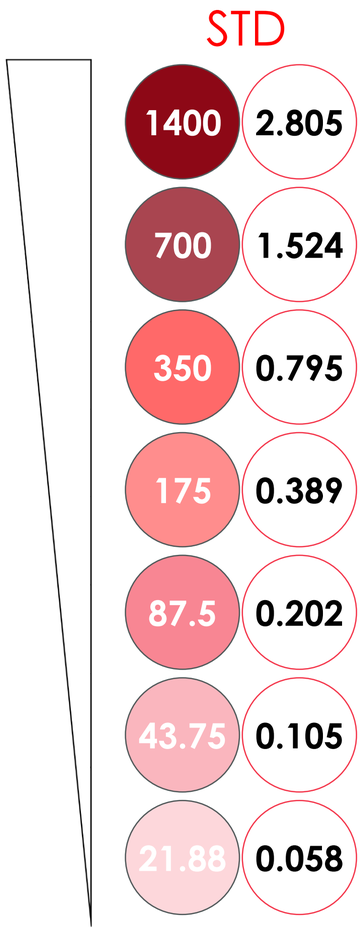


4 parameters model (4PL)

Software of your reader,
GraphPad PRISM

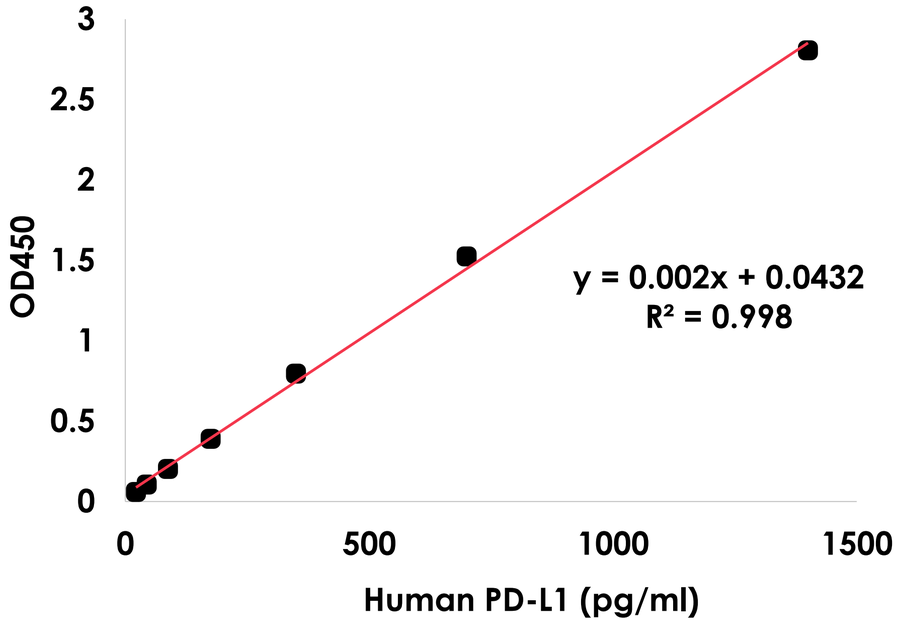
Linear regression

Excel

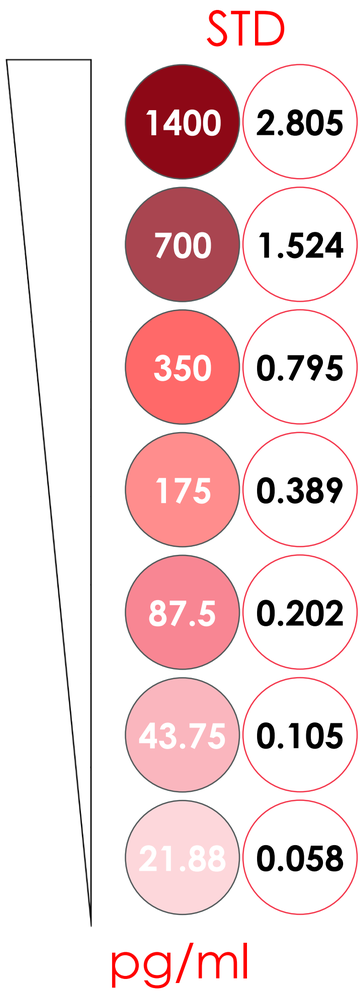


Excel

Normal scale

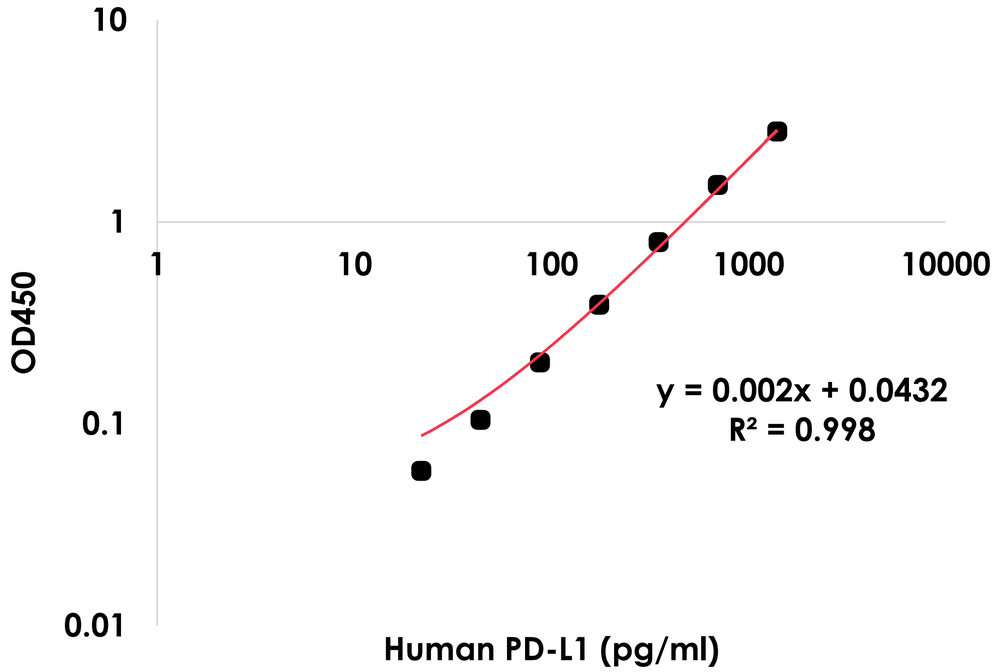


Linear regression



Excel

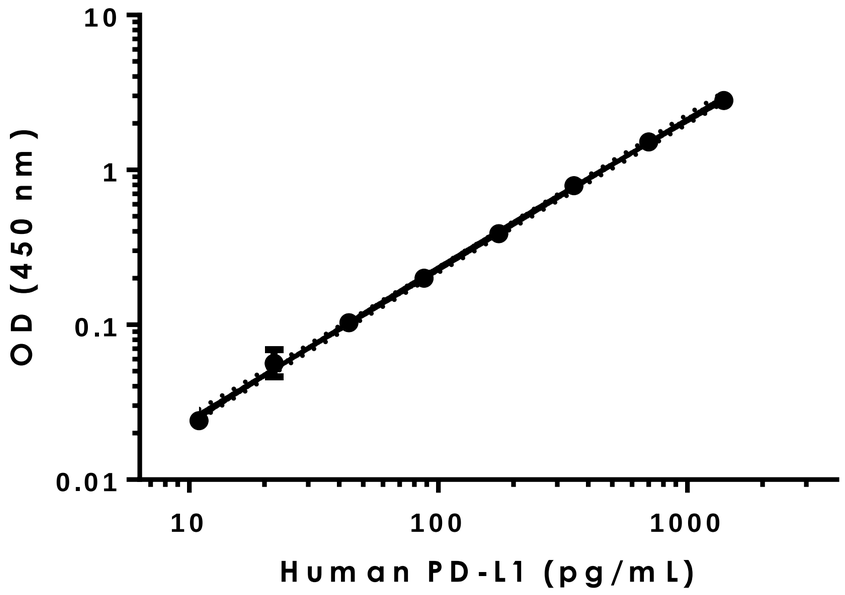
Log/log scale



Linear regression

GraphPad PRISM

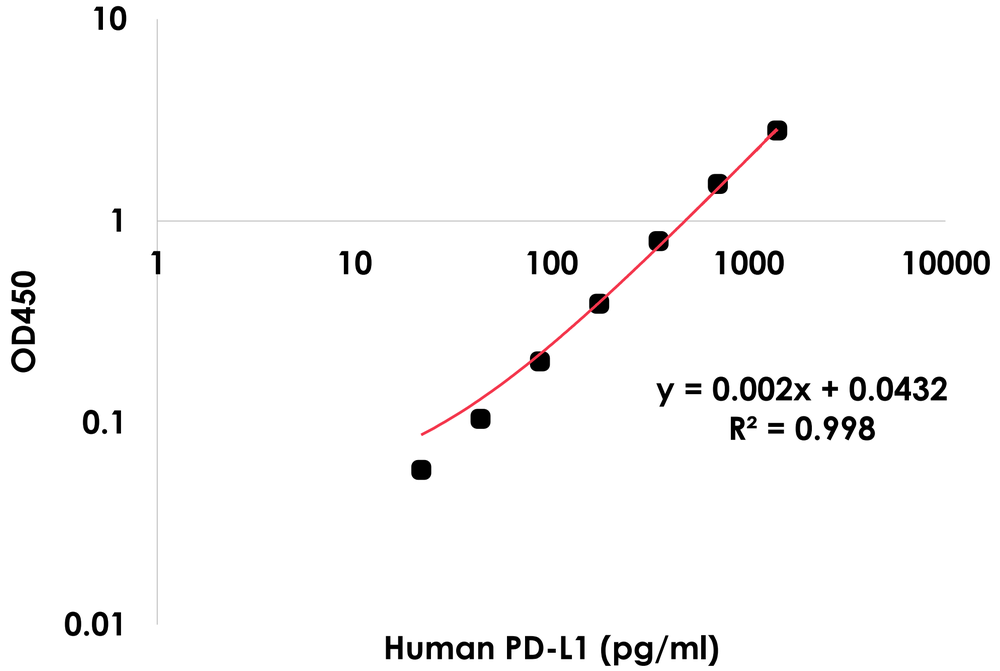
Log/log scale



4 parameters model (4PL)

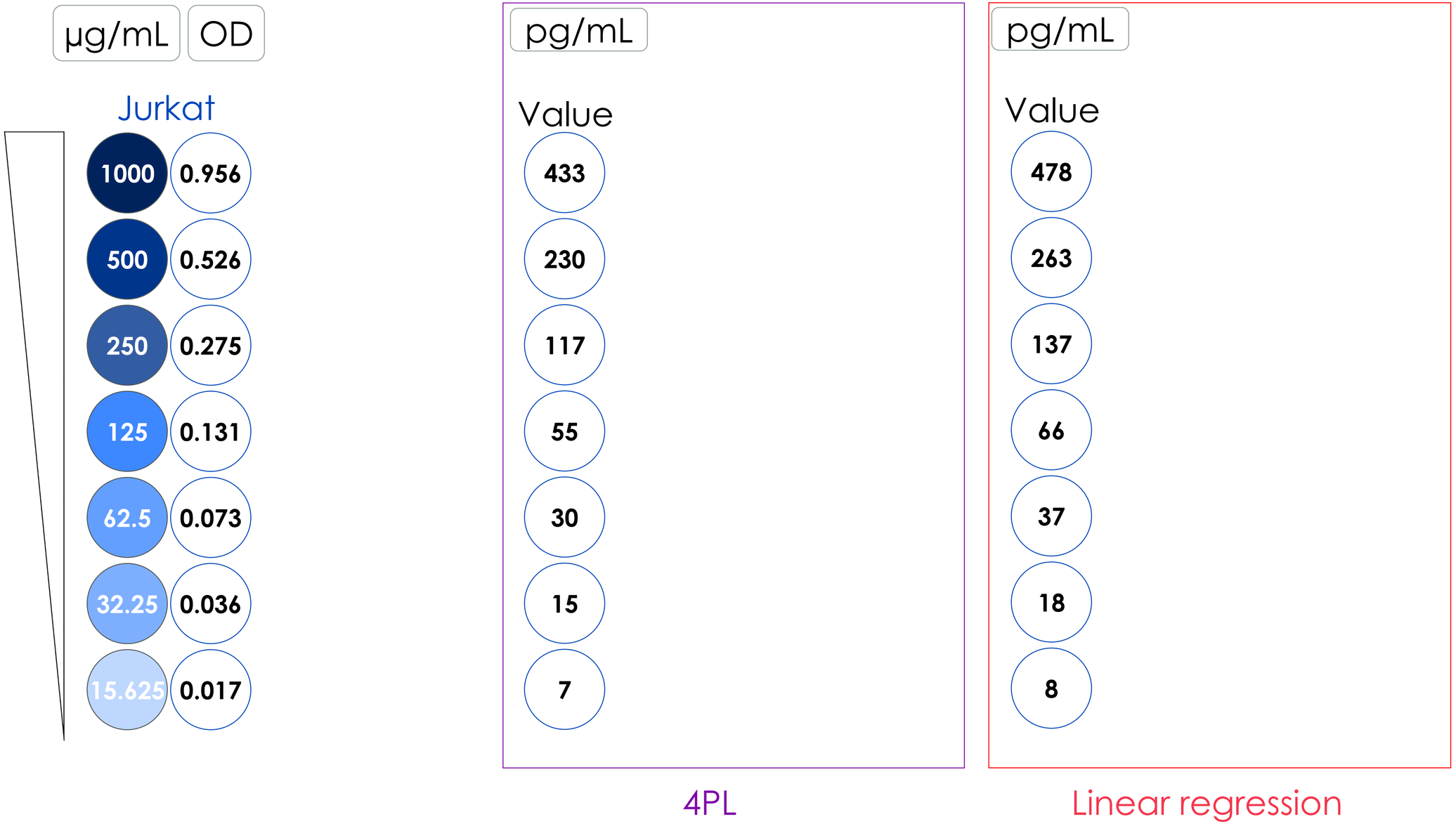
Excel

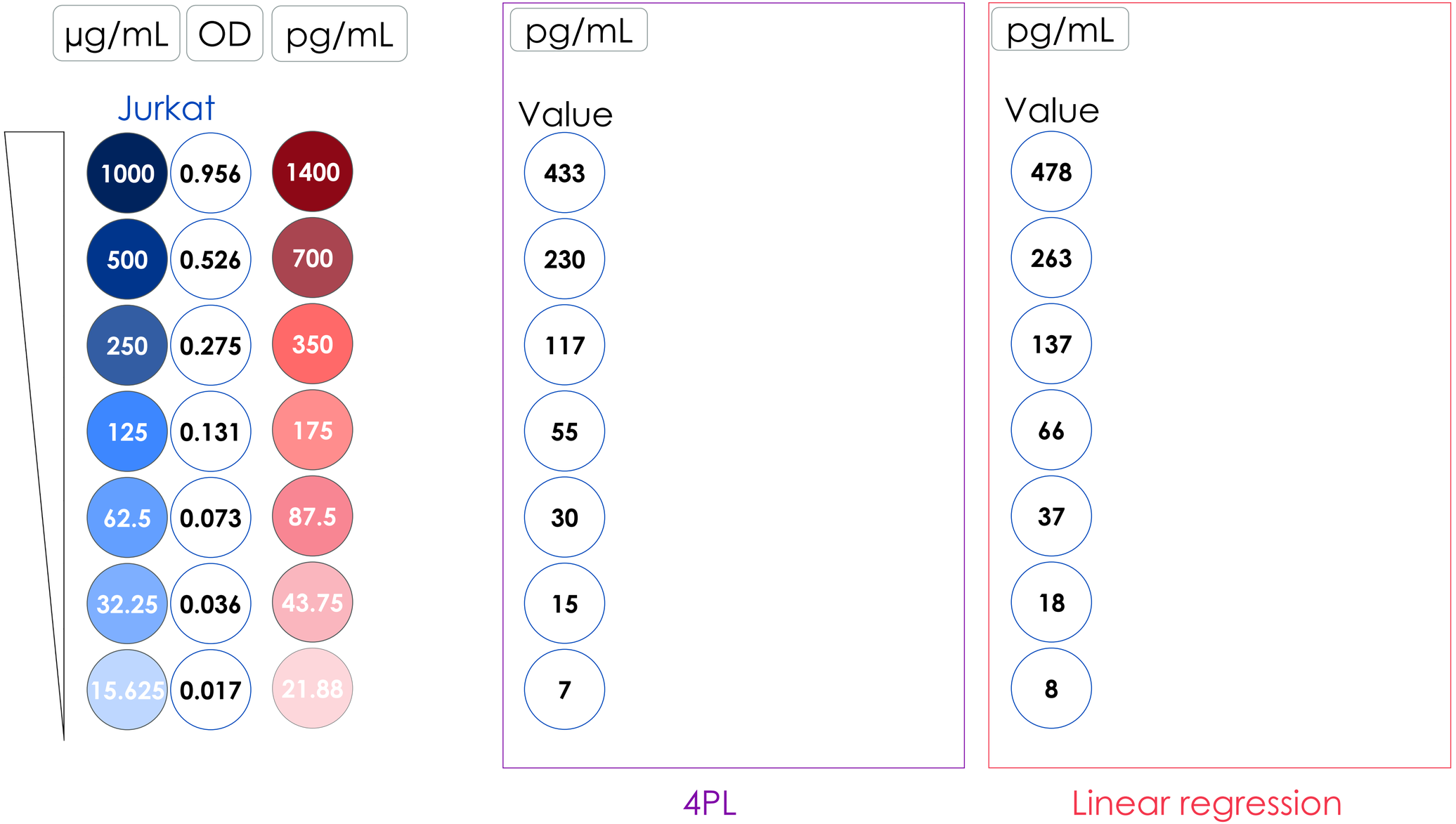
Log/log scale



Linear regression

Calculating the concentration of your samples


µg/mL
OD
Jurkat
1000
0.956
500
0.526
250
0.275
125
0.131
62.5
0.073
32.25
0.036
15.625
0.017
pg/mL
Value
433
230
117
55
30
15
7
4PL
pg/mL
Value
478
263
137
66
37
18
8
Linear regression


µg/mL
OD
pg/mL
Jurkat
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0.526
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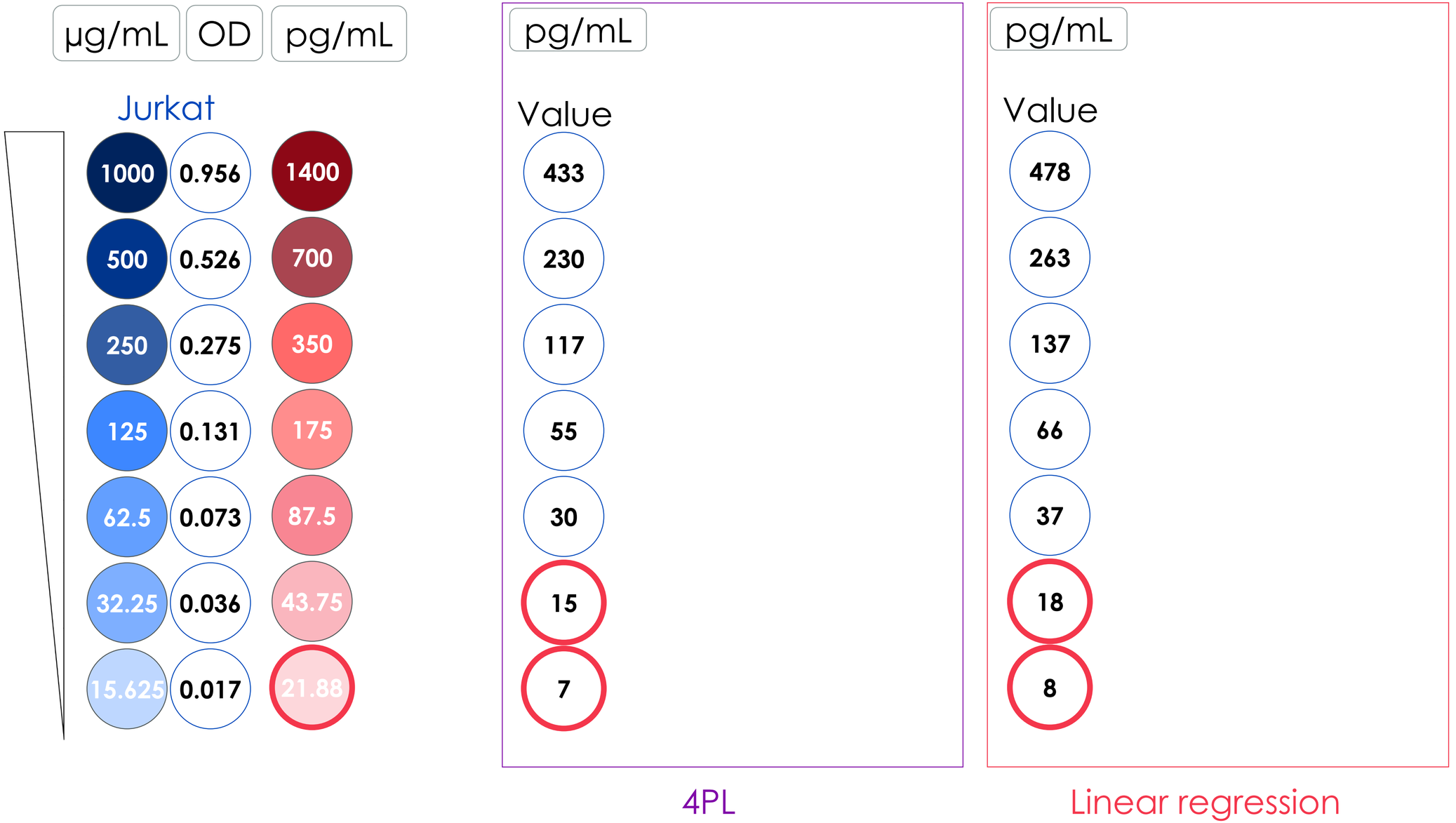
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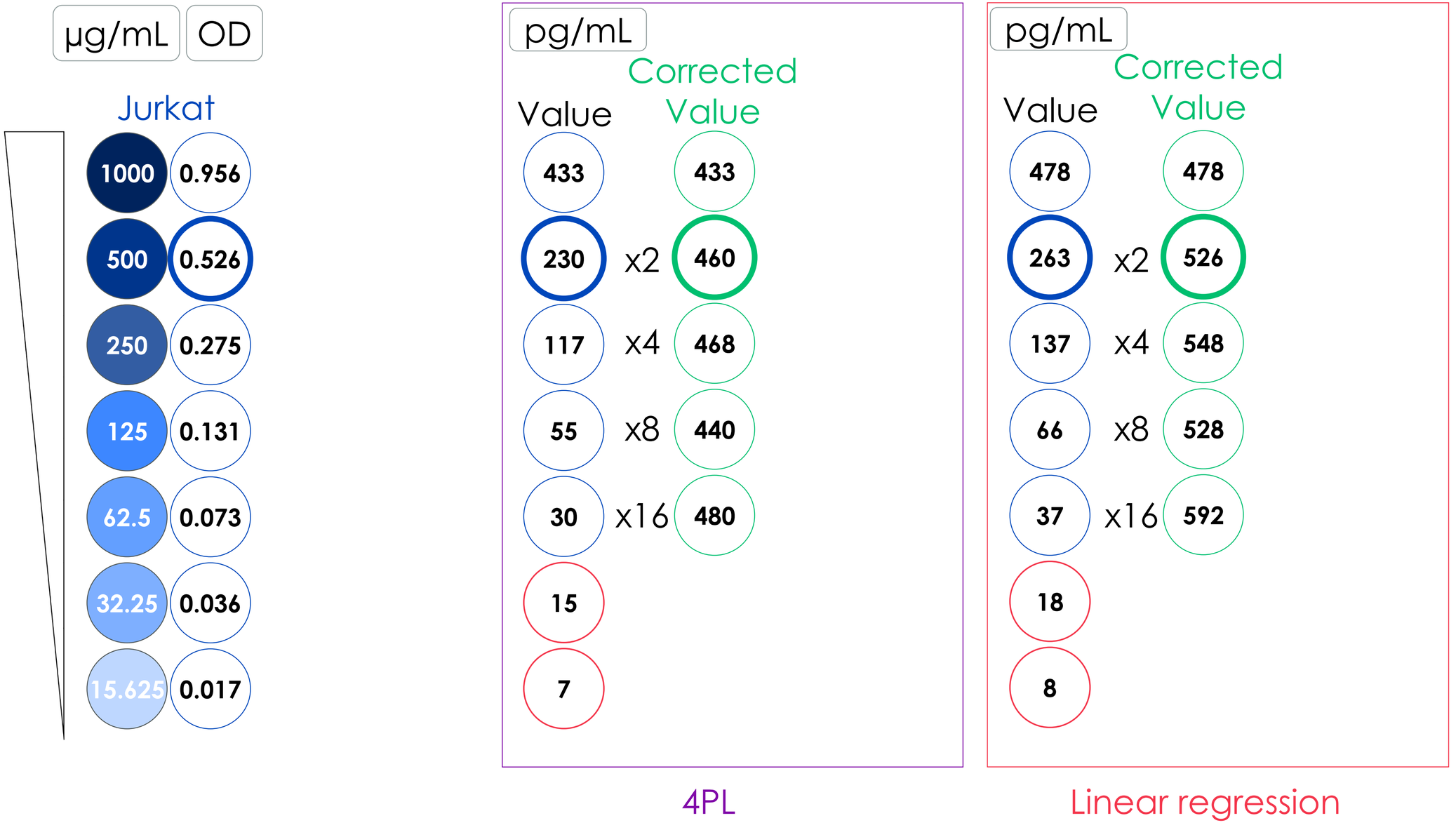
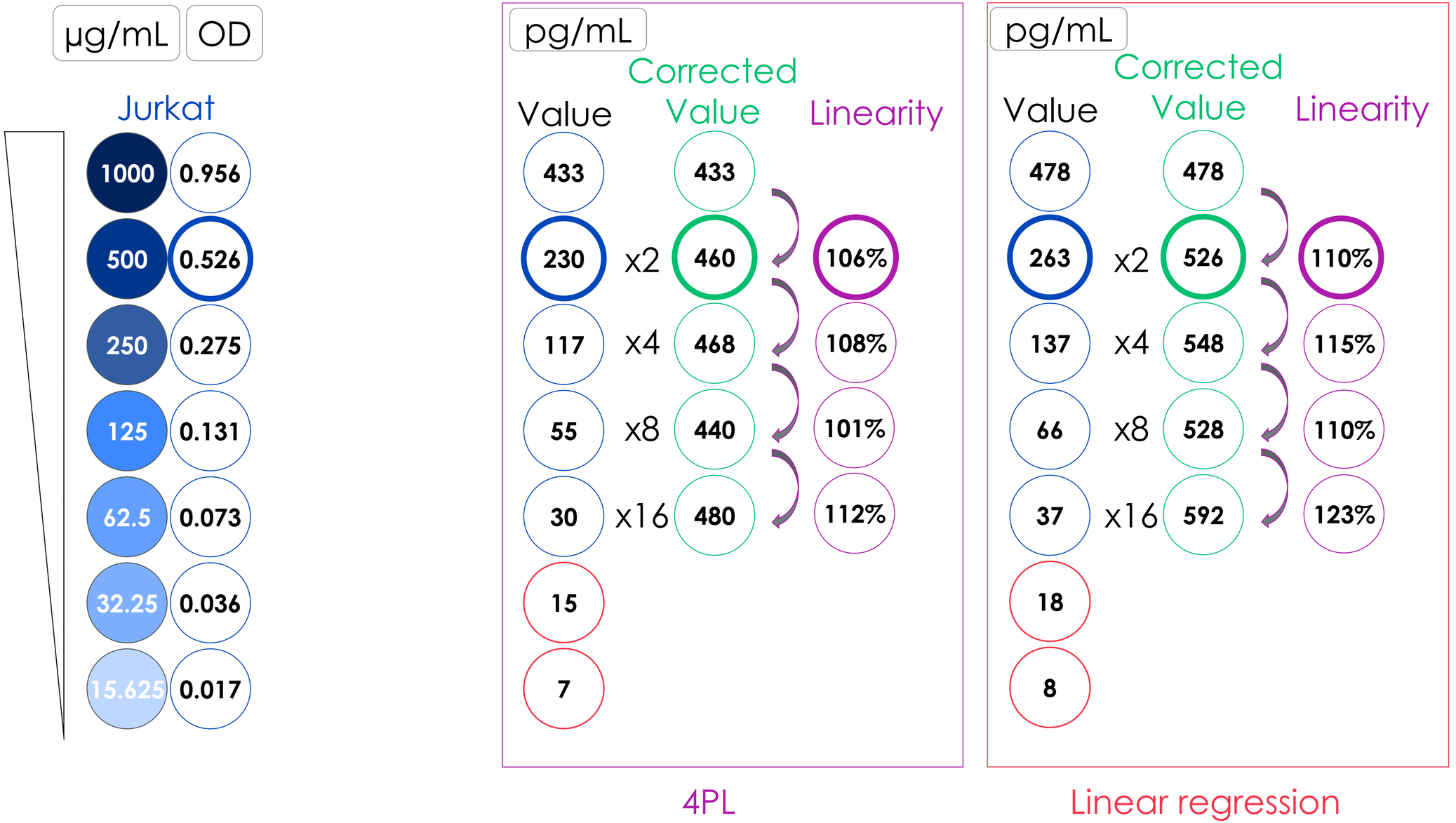
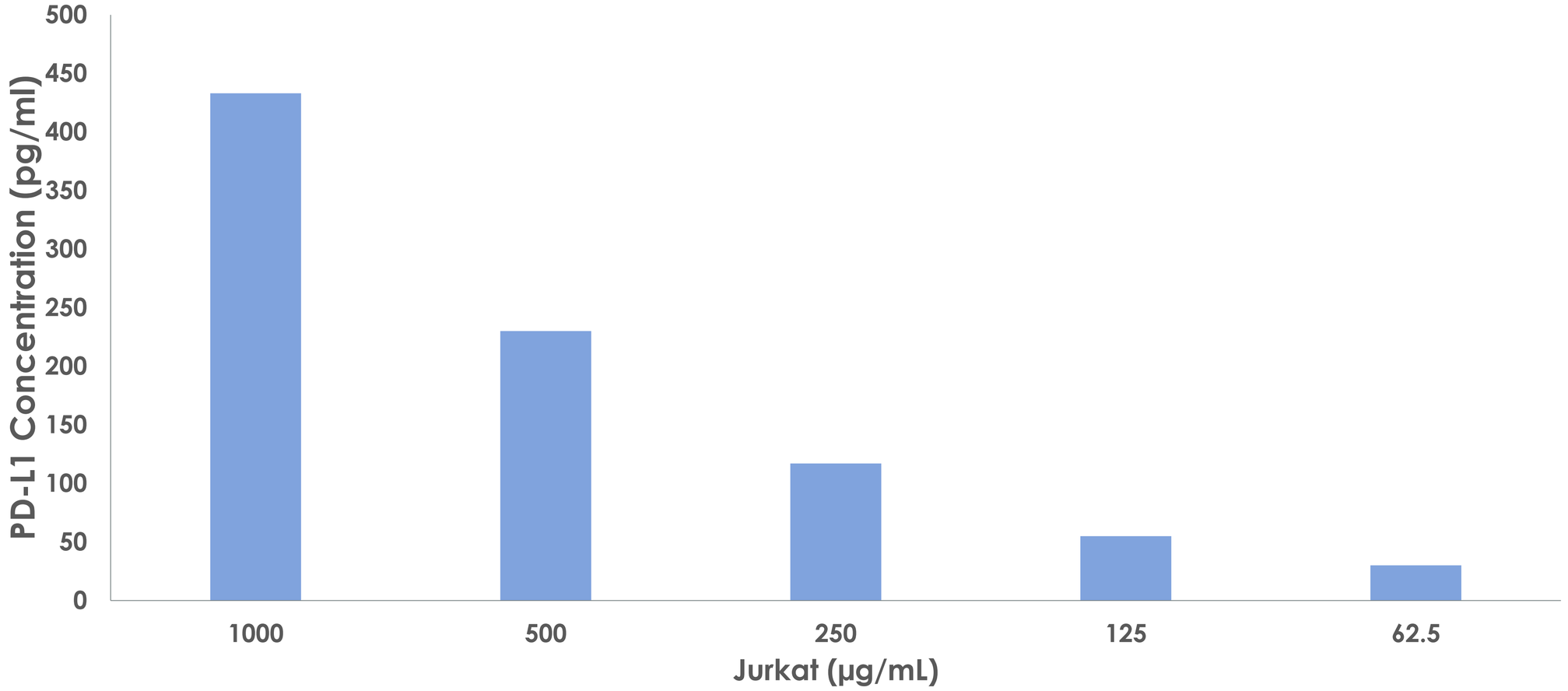

µg/mL
OD
Jurkat
1000
0.956
500
0.526
250
0.275
125
0.131
62.5
0.073
32.25
0.036
15.625
0.017
pg/mL
Value
Corrected Value
433
433
230
x2
460
117
x4
468
55
x8
440
30
x16
480
15
7
4PL
pg/mL
Value
Corrected Value
478
478
263
x2
526
137
x4
548
66
x8
528
37
x16
592
18
8
Linear regression


µg/mL
OD
Jurkat
1000
0.956
500
0.526
250
0.275
125
0.131
62.5
0.073
32.25
0.036
15.625
0.017
pg/mL
Corrected
Value
Value
Linearity
433
433
230
x2
460
106%
117
x4
468
108%
55
x8
440
101%
30
x16
480
112%
15
7
4PL
pg/mL
Corrected
Value
Value
Linearity
478
478
263
x2
526
110%
137
x4
548
115%
66
x8
528
110%
37
x16
592
123%
18
8
Linear regression

SimpleStep ELISA®

Human PD-L1 ELISA Kit [28-8] (ab214565)



Human PD-L1 ELISA Kit [28-8] (ab214565)

Typical Sample Dynamic Range	
Sample Type	**Range**
Human Plasma - Heparin	1.56 – 50%
Human Plasma - EDTA	1.56 – 50%
Human Plasma - Citrate	1.56 – 50%
Human Serum	1.56 – 50%
Human Urine	1.56 – 50%
Cell Culture Supernatant	Varies by type
Jurkat LPS+IFN-gamma Cell Extract	31.25 – 1,000 µg/mL
Placenta Tissue Extract	31.25 – 1,000 µg/mL

Contact us!

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French supportscientifique@abcam.com

Spanish soportecientifico@abcam.com

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