

25(OH) Vitamin D ELISA ADI-900-215 ASSAY LAYOUT SHEET

• **TABLE FOR DISCRETE STANDARDS 1-6:**

Standard	25(OH) Vitamin D ₃ Vial Concentration (ng/mL)	25(OH) Vitamin D ₃ In-Well Concentration (ng/mL)
1	1010	101
2	279	27.9
3	71.6	7.16
4	24.4	2.44
5	4.8	0.48
6	0.5	0.05

• **ASSAY PROTOCOL FLOW CHART: Volume Added (µL)**

	NSB	Bo	Standards	Samples
Well ID:	A1, B1	C1, D1	G1-D3	E3-H12
Dissociation Buffer	90 µL	90 µL	90 µL	90 µL
Sample Diluent	10 µL	10 µL	---	---
Standards or Samples	---	---	10 µL	10 µL
Incubate 5 min at RT with shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
25(OH) Vitamin D ₃ Conjugate, 1X	50 µL	50 µL	50 µL	50 µL
25(OH) Vitamin D ₃ Conjugate Diluent	50 µL	---	---	---
25(OH) Vitamin D ₃ Antibody	---	50 µL	50 µL	50 µL
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x Full well vol. (~325µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
pNPP substrate	200 µL	200 µL	200 µL	200 µL
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 µL	50 µL	50 µL	50 µL
Read OD at 405 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

25(OH) Vitamin D PLATE LAYOUT:

A1 NSB	A2 Std 3	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 NSB	B2 Std 3	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 Bo	C2 Std 4	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Bo	D2 Std 4	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std 1	E2 Std 5	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std 1	F2 Std 5	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std 2	G2 Std 6	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 2	H2 Std 6	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. _____ Exp. Date _____ Date _____ Tech. _____

1st Incub.: Start Time _____ Temp. _____ 3rd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____ End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____