

human Osteopontin ASSAY LAYOUT SHEET for use with Assay Designs' Catalog No. 900-142

• TABLE FOR MAKING STANDARDS 1-5*

* Standards should be diluted in polypropylene test tubes and used within 60 minutes of preparation.

Std.	Assay Buffer 10 plus Inhibitors Vol. (μL)	Standard Vol. Added (μL)	human OPN Conc. (ng/mL)
1	500	lyophilized	32
2	250	250, Std. 1	16
3	250	250, Std. 2	8
4	250	250, Std. 3	4
5	250	250, Std. 4	2

• ASSAY PROTOCOL FLOW CHART

Well ID:	Blank A1, B1	S0 C1-D1	Standards E1-F2	Samples G2-H12
Assay Buffer 10, plus Inhibitors	---	100 μL	---	---
Std. or Sample	---	---	100 μL	100 μL
Seal plate and tap gently to mix	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. 1 hour @ RT, shaking and sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Antibody	---	100 μL	100 μL	100 μL
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	100 μL	100 μL	100 μL
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
pNpp Substrate	100 μL	100 μL	100 μL	100 μL
Incub. 30 min. @ RT, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution (TSP)	25 μL	25 μL	25 μL	25 μL
Read OD at 405 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

human OPN PLATE LAYOUT:

A1 Blank	A2 Std 3	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 3	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 S0	C2 Std 4	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 S0	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	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 2	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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

1st Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

3rd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

4th Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____