

BIG ENDOTHELIN-1 (HUMAN) ASSAY LAYOUT SHEET
for use with Enzo Life Sciences Catalog No. ADI-900-022

• **DILUTION TABLE FOR MAKING STANDARDS 1-8:**

* First add 500 µL of DI water to the vial of Standard, let sit for 5 min., then mix gently.

Std.	Assay Buffer Vol. (µL)	Vol. Added (µL)	h Big ET-1 Conc. (pg/mL)
1	220	220, Stock	100
2	220	220, Std. 1	50
3	220	220, Std. 2	25
4	220	220, Std. 3	12.5
5	220	220, Std. 4	6.25
6	220	220, Std. 5	3.13
7	220	220, Std. 6	1.56
8	220	220, Std. 7	0.78

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	S0 C1, D1	Stds. E1 - D3	Samples E3 - H12
Assay Buffer	---	100 µL	---	---
Std. and/or Sample	---	---	100 µL	100 µL
Tap plate gently & seal	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. overnight @ 4 °C	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 7 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Labeled Antibody	---	100 µL	100 µL	100 µL
Seal plate & Incub. 30 min. @ 4 °C	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 9 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Substrate	100 µL	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, dark	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	100 µL	100 µL	100 µL	100 µL

BIG ENDOTHELIN-1 (HUMAN) PLATE LAYOUT:

A1 Blank	A2 Std 3	A3 Std 7	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 3	B3 Std 7	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 S0	C2 Std 4	C3 Std 8	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 S0	D2 Std 4	D3 Std 8	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. _____ Notes: _____

End Time _____ Temp. _____ Notes: _____

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

End Time _____ Temp. _____ Notes: _____