

DIRECT 8-iso-PGF_{2α} ASSAY LAYOUT SHEET

Use with The Enzo Life Sciences Catalog Nos. ADI-900-091 and ADI-901-091

• **DILUTION TABLE FOR MAKING STANDARDS 1-5:**

Std.	Direct 8-iso Sample Diluent Vol. (μL)	Vol. Added (μL)	8-iso-PGF _{2α} Conc. (pg/mL)
1	900	100, Stock	100,000
2	800	200, Std. 1	20,000
3	800	200, Std. 2	4,000
4	800	200, Std. 3	800
5	800	200, Std. 4	160

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2 - B3	Samples C3 - H12
Neutralizing Reagent	---	---	50μL	50μL	50μL	50μL
Direct 8-iso Sample Diluent	---	---	100μL	50μL	---	---
Stds and/or Samples	---	---	---	---	50μL	50μL
Conjugate	---	---	50μL	50μL	50μL	50μL
Antibody	---	---	---	50μL	50μL	50μL
Incub. 2 hours @ RT, shaking or 18-24 hours @ 4°C	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 400μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	5μL*	---	---	---	---
Substrate	200μL	200μL	200μL	200μL	200μL	200μL
Incub. 45 min. @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50μL	50μL	50μL	50μL	50μL	50μL

* Conjugate for TA must be diluted 1:10 in Assay Buffer. Add 100μL of A.B. to a clean test tube. From this volume remove 10μL of A.B and, using the same measuring device, add 10 uL of Direct 8-iso PGF_{2α} Conjugate

• **DIRECT 8-iso-PGF_{2α} PLATE LAYOUT:**

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

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____