

17 β -ESTRADIOL (SERUM/PLASMA) ASSAY LAYOUT SHEET
for use with Enzo Life Sciences Catalog Nos. ADI-900-174 & ADI-901-174

• **DILUTION TABLE FOR MAKING STANDARDS 1-7:**

Std.	Assay Buffer Vol. (μL)	Vol. Added (μL)	17β-Estradiol Conc. (pg/mL)
Int.	990	10, Stock	3000
1	800	400, Int.	1000
2	500	500, Std. 1	500
3	500	500, Std. 2	250
4	500	500, Std. 3	125
5	500	500, Std 4	62.5
6	500	500, Std 5	31.3
7	500	500, Std 6	15.6

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2-F3	Samples G3-H12
Assay Buffer	---	---	150 μ L	100 μ L	---	---
Std. and/or Sample	---	---	---	---	100 μ L	100 μ L
Conjugate	---	---	50 μ L	50 μ L	50 μ L	50 μ L
Antibody	---	---	---	50 μ L	50 μ L	50 μ L
Incub. 2 hrs. @ RT, shaking (sealed)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	5 μ L*	---	---	---	---
Substrate	200 μ L	200 μ L	200 μ L	200 μ L	200 μ L	200 μ L
Incub. 1 hour @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L	50 μ L
read @ 405 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

• **17 β -ESTRADIOL 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 Std 6	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 TA	D2 Std 2	D3 Std 6	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 NSB	E2 Std 3	E3 Std 7	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 NSB	F2 Std 3	F3 Std 7	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. _____