

## 17 $\beta$ -ESTRADIOL ASSAY LAYOUT SHEET

for use with Enzo Life Sciences Catalog Nos. ADI-900-008 & ADI-901-008

• **DILUTION TABLE FOR MAKING STANDARDS 1-6:**

Std.	Standard Diluent Vol. ( $\mu$ L)	Vol. Added ( $\mu$ L)	17 $\beta$ -Estradiol Conc. (pg/mL)
1	900	100, Stock	30,000
2	750	250, Std. 1	7,500
3	750	250, Std. 2	1,875
4	750	250, Std. 3	468.8
5	750	250, Std. 4	117.2
6	750	250, Std. 5	29.3

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2 - D3	Samples E3 - H12
Standard Diluent	---	---	100 $\mu$ L	100 $\mu$ L	---	---
Assay Buffer 3	---	---	50 $\mu$ L	---	---	---
Std. and/or Sample	---	---	---	---	100 $\mu$ L	100 $\mu$ L
Conjugate	---	---	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L
Antibody	---	---	---	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L
Incub. 2 hours @ RT, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 200 $\mu$ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	5 $\mu$ L	---	---	---	---
Substrate	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L
Incub. 45 min. @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L	50 $\mu$ L

• **17 $\beta$ -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	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. \_\_\_\_\_ \_\_\_\_\_