

ESTRIOL ASSAY LAYOUT SHEET

for use with Enzo Life Sciences Catalog Nos. ADI-900-100 & ADI-901-100

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

| Std. | Standard Diluent Vol. (µL) | Vol. Added (µL) | Estriol Conc. (pg/mL) |
|------|-------------------------------|--------------------|--------------------------|
| 1 | 900 | 100, Stock | 500,000 |
| 2 | 750 | 250, Std. 1 | 125,000 |
| 3 | 750 | 250, Std. 2 | 31,250 |
| 4 | 750 | 250, Std. 3 | 7,812 |
| 5 | 750 | 250, Std. 4 | 1,953 |
| 6 | 750 | 250, Std. 5 | 488 |
| 7 | 750 | 250, Std. 6 | 122 |

• **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 |
|------------------------------|-----------------|--------------|---------------|--------------|------------------|---------------------|
| Standard Diluent | --- | --- | 100 µL | 100 µL | --- | --- |
| Assay Buffer | --- | --- | 50 µL | --- | --- | --- |
| Std. and/or Sample | --- | --- | --- | --- | 100 µL | 100 µL |
| Conjugate | --- | --- | 50 µL | 50 µL | 50 µL | 50 µL |
| Antibody | --- | --- | --- | 50 µL | 50 µL | 50 µL |
| Incub. 2 hours @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 3 x 400 µL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Conjugate | --- | 5 µL | --- | --- | --- | --- |
| Substrate | 200 µL | 200 µL | 200 µL | 200 µL | 200 µL | 200 µL |
| Incub. 1 hour @ RT | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Stop Solution | 50 µL | 50 µL | 50 µL | 50 µL | 50 µL | 50 µL |

• **ESTRIOL 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. _____