

## DHEA ASSAY LAYOUT SHEET

for use with Enzo Life Sciences Catalog Nos. ADI-900-093 and ADI-901-093

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

| Std. | Diluent Vol. (µL) | Vol. Added (µL) | DHEA Conc. (pg/mL) |
|------|-------------------|-----------------|--------------------|
| 1    | 900               | 100, Stock      | 50,000             |
| 2    | 750               | 250, Std. 1     | 12,500             |
| 3    | 750               | 250, Std. 2     | 3,125              |
| 4    | 750               | 250, Std. 3     | 781.25             |
| 5    | 750               | 250, Std. 4     | 195.31             |
| 6    | 750               | 250, Std. 5     | 48.83              |
| 7    | 750               | 250, Std. 6     | 12.21              |

• **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                     | ---          | <u>5 µL</u> * | ---        | ---       | ---           | ---              |
| Substrate                     | 200 µL       | 200 µL        | 200 µL     | 200 µL    | 200 µL        | 200 µL           |
| Incub. 3 hours @ 37°C, sealed | ⇒⇒⇒⇒         | ⇒⇒⇒⇒          | ⇒⇒⇒⇒       | ⇒⇒⇒⇒      | ⇒⇒⇒⇒          | ⇒⇒⇒⇒             |
| Stop Solution                 | 50 µL        | 50 µL         | 50 µL      | 50 µL     | 50 µL         | 50 µL            |

\* Conjugate for TA must be diluted 1:10 in Standard diluent. Add 500 µL of Standard diluent to a clean test tube. From this volume, remove 50 µL of Standard diluent. and, using the same measuring device, add 50 µL of DHEA Conjugate.

• **DHEA 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. \_\_\_\_\_