

**IL-6 (MOUSE) ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences Catalog No. ADI-900-045

• **TABLE FOR MAKING STANDARDS 1-8:**

**NOTE:** Reconstitute lyophilized standard with 2 mL standard diluent for Std. 1.

| Std. | Diluent Vol. (µL) | Vol. Added (µL) | mouse IL-6 Conc. (pg/mL) |
|------|-------------------|-----------------|--------------------------|
| 1    | 2000              | (lyoph.)        | 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      | 31.25                    |
| 7    | 500               | 500, Std 6      | 15.62                    |
| 8    | 500               | 500, Std. 7     | 7.81                     |

• **ASSAY PROTOCOL FLOW CHART:**

| Well ID:                               | Blank<br>A1, B1 | S0<br>C1, D1 | Standards<br>E1-D3 | Samples<br>E3-H12 |
|--|-----------------|--------------|--------------------|-------------------|
| Standard Diluent                       | ---             | 50µL         | ---                | ---               |
| Std. and/or Sample                     | ---             | ---          | 50µL               | 50µL              |
| Tap plate gently to mix                | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Incub. 1 hr. @ RT, sealed, shaking     | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400µL                  | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Antibody                               | ---             | 50µL         | 50µL               | 50µL              |
| Incub. 1hr @ RT, sealed, shaking       | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400µL                  | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Conjugate                              | ---             | 50µL         | 50µL               | 50µL              |
| Incub. 30 min. @ RT, sealed, shaking   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400µL                  | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| TMB Substrate                          | 50µL            | 50µL         | 50µL               | 50µL              |
| Incub. uncovered 15 min. @ RT, shaking | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Stop Solution 2 (1N HCl)               | 50µL            | 50µL         | 50µL               | 50µL              |
| Read OD at 450nm                       | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |

**IL-6 (MOUSE) PLATE LAYOUT:**

|          |          |          |    |    |    |    |    |    |     |     |     |
|----------|----------|----------|----|----|----|----|----|----|-----|-----|-----|
| A1 Blank | A2 Std 3 | A3 Std 7 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 Blank | B2 Std 3 | B3 Std 7 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 S0    | C2 Std 4 | C3 Std 8 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 S0    | D2 Std 4 | D3 Std 8 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 Std 1 | E2 Std 5 | E3       | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 Std 1 | F2 Std 5 | F3       | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 Std 2 | G2 Std 6 | G3       | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 Std 2 | H2 Std 6 | H3       | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

Kit Lot No. \_\_\_\_\_ Exp. Date \_\_\_\_\_ Date \_\_\_\_\_ Tech. \_\_\_\_\_

1<sup>st</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

3<sup>rd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_

2<sup>nd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

4<sup>th</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_