# LEPTIN (RAT) ASSAY LAYOUT SHEET
## for use with Enzo Life Sciences Catalog No. ADI-900-015A

### • DILUTION TABLE FOR MAKING STANDARDS:

| Std. | Assay Buffer 28 Vol. (µL) | Vol. Added (µL) | Mouse Leptin Conc. (pg/mL) |
|---|---|---|---|
| 1 | 1000 | Lyoph Std | 6,400 |
| 2 | 500 | 500, Std. 1 | 3,200 |
| 3 | 500 | 500, Std. 2 | 1,600 |
| 4 | 500 | 500, Std. 3 | 800 |
| 5 | 500 | 500, Std. 4 | 400 |
| 6 | 500 | 500, Std. 5 | 200 |
| 7 | 500 | 500, Std. 6 | 100 |

### • ASSAY PROTOCOL FLOW CHART:

| Well ID: | Blank A1, B1 | S0 C1, D1 | Standards E1-B3 | Samples C3-H12 |
|---|---|---|---|---|
| Assay Buffer | ––– | 100µL | ––– | ––– |
| Std. and/or Sample | ––– | ––– | 100µL | 100µL |
| Tap plate gently to mix | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Incub. 1 hour @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 3 x 400µL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Antibody | ––– | 100µL | 100µL | 100µL |
| Incub. 1 hour @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 3 x 400µL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Conjugate | ––– | 100µL | 100µL | 100µL |
| Incub. 30 min. @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 3 x 400µL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| TMB Substrate | 100µL | 100µL | 100µL | 100µL |
| Incub. 30 min. @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Stop Solution 2 (1N HCl) | 100µL | 100µL | 100µL | 100µL |
| Read OD at 450nm | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |


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## • LEPTIN (RAT) 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 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 S0 | D2 Std 4 | D3 | 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. _____

1st Incub.: Start Time _____ Temp. _____ 3rd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____ End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____ 4th Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____ End Time _____ Temp. _____


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