

**Total PSA ELISA ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences' Catalog No. ENZ-KIT146-0001

• **TABLE FOR MAKING STANDARDS 1-6:**

| Std. | AB41<br>Vol. (µL) | Standard<br>Vol. Added (µL) | Total PSA<br>Conc. (pM) |
|------|-------------------|-----------------------------|-------------------------|
| 1    | 475               | 25, stock                   | 333                     |
| 2    | 300               | 150, Std. 1                 | 111                     |
| 3    | 300               | 150, Std. 2                 | 37                      |
| 4    | 300               | 150, Std. 3                 | 12.33                   |
| 5    | 300               | 150, Std. 4                 | 4.11                    |
| 6    | 300               | 150, Std. 5                 | 1.37                    |

• **ASSAY PROTOCOL FLOW CHART: Vol. Added (µL)**

| Well ID:                                | Blank<br>A1, B1 | NSB<br>C1, D1 | S0<br>E1-F1 | Standards<br>G1-B3 | Samples<br>C3-H12 |
|---|-----------------|---------------|-------------|--------------------|-------------------|
| Assay Buffer 41                         | ---             | 100 µL        | 100 µL      | ---                | ---               |
| Standards or Samples                    | ---             | ---           | ---         | 100 µL             | 100 µL            |
| Incub. 30 min. @ RT, sealed & shaking   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x Full well vol. (~400µL) | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Assay Buffer 41                         | ---             | 100 µL        | ---         | ---                | ---               |
| Total PSA Detector Antibody             | ---             | ---           | 100 µL      | 100 µL             | 100 µL            |
| Incub. 60 min. @ RT, sealed & shaking   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x Full well vol. (~400µL) | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Total PSA Conjugate                     | ---             | 100 µL        | 100 µL      | 100 µL             | 100 µL            |
| Incub. 30 min. @ RT, sealed & shaking   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x Full well vol. (~400µL) | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| TMB Substrate                           | 100 µL          | 100 µL        | 100 µL      | 100µL              | 100 µL            |
| Incub. 30 min. @ RT, sealed & shaking   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Stop Solution 2 (1N HCl)                | 100 µL          | 100 µL        | 100 µL      | 100 µL             | 100 µL            |
| Read OD at 450 nm                       | ⇒⇒⇒⇒            | ⇒⇒⇒⇒          | ⇒⇒⇒⇒        | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |

**Total PSA PLATE LAYOUT:**

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