

[pSer473/474]Akt1/2 ELISA ASSAY LAYOUT SHEET

for use with Enzo Life Sciences Catalog No. ADI-900-162

• **TABLE FOR MAKING STANDARDS 1-6:**

NOTE: Reconstitute lyophilized Akt1 (phospho-Ser473) Standard with 1 mL standard diluent (this is Standard 1).

| Std. | Diluent Vol. (μL) | Vol. Added (μL) | Akt1 (phospho-Ser473) Conc. (pg/mL) |
|------|-------------------|-----------------|-------------------------------------|
| 1 | 1,000 | (lyoph.) | 560 |
| 2 | 500 | 500, Std. 1 | 280 |
| 3 | 500 | 500, Std. 2 | 140 |
| 4 | 500 | 500, Std. 3 | 70 |
| 5 | 500 | 500, Std. 4 | 35 |
| 6 | 500 | 500, Std. 5 | 17.5 |

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

| Well ID: | Blank A1, B1 | S0 C1-D1 | Standards E1-H2 | Samples A3-H12 |
|---------------------------------------|-----------------|-------------|--------------------|-------------------|
| Standard Diluent | --- | 100 μL | --- | --- |
| Std. or Sample | --- | --- | 100 μL | 100 μL |
| Seal plate and tap gently to mix | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Incub. 1 hour @ RT, shaking & sealed | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 5 x 400 μL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Antibody | --- | 100 μL | 100 μL | 100 μL |
| Incub. 1 hour @ RT, shaking & sealed | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 5 x 400 μL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Conjugate | --- | 100 μL | 100 μL | 100 μL |
| Incub. 30 min. @ RT, shaking & sealed | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Asp. & Wash 5 x 400 μL | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| TMB Substrate | 100 μL | 100 μL | 100 μL | 100 μL |
| Incub. 30 min. @ RT, shaking | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |
| Stop Solution 2 (1N HCl) | 100 μL | 100 μL | 100 μL | 100 μL |
| Read OD at 450 nm | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ | ⇒⇒⇒⇒ |

[pSer473/474]Akt1/2 PLATE LAYOUT:

| | | | | | | | | | | | |
|----------|----------|----|----|----|----|----|----|----|-----|-----|-----|
| A1 Blank | A2 Std 3 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 Blank | B2 Std 3 | B3 | 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. _____
 End Time _____ Temp. _____

3rd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

4th Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____