

**[pSer15]HSP27 (human) ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences Catalog No. ADI-900-170

• **TABLE FOR MAKING STANDARDS 1-6:**

<b>Std.</b>	<b>Diluent Vol. (µL)</b>	<b>Vol. Added (µL)</b>	<b>Hsp27 (phospho-Ser15) Conc. (pg/mL)</b>
1	900	100, Stock	1,000
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

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

<b>Well ID:</b>	<b>Blank A1, B1</b>	<b>S0 C1-D1</b>	<b>Standards E1-H2</b>	<b>Samples A3-H12</b>
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 6 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Antibody	---	100 µL	100 µL	100 µL
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 6 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 6 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
TMB Substrate	100 µL	100 µL	100µL	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	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

**Hsp27 (phospho-Ser15) 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. \_\_\_\_\_

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. \_\_\_\_\_