

[pSer326] HSF1 ASSAY LAYOUT SHEET
for use with Enzo Life Sciences Catalog No. ADI-900-199

• **TABLE FOR MAKING STANDARDS 1-6:**

Std.	Diluent Vol. (µL)	Frozen Stock/Standard Vol. Added (µL)	Phosphorylated Conc. (ng /mL)
1	250	250	12.5
2	250	200, Std. 1	6.25
3	250	250, Std. 2	3.125
4	250	250, Std. 3	1.56
5	250	250, Std. 4	0.78
6	250	250, Std. 5	0.39

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

	Blank	S0	Standards	Samples
Well ID:	A1, B1	C1-D1	E1-H2	C1-H12
Standard Diluent	---	100 µL	---	---
Std and/or Sample	---	---	100 µL	100 µL
Seal plate and tap gently to mix	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. 1 hour @ RT, shaking, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
[pSer326] HSF1 Antibody	---	100 µL	100 µL	100 µL
Incub. 1 hour @ RT, shaking,, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
SA-HRP Conjugate	---	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, shaking, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 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	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

[pSer326] HSF1 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 Std 0	C2 Std 4	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Std 0	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. _____