

## HUMAN TOTAL SURVIVIN ASSAY LAYOUT SHEET for use with Assay Designs' Catalog No. 900-111

### • TABLE FOR MAKING STANDARDS 1-6\*

\*Allow the 500 pg/vial Survivin Standard to warm for **not more than 10 minutes**. Reconstitute lyophilized standard with 500  $\mu\text{L}$  of Standard Diluent (Assay Buffer 20 plus Inhibitors or Tissue Culture Media) for Standard 1. Let sit 5 minutes, then mix gently. **Diluted standards should be used within 20 minutes of preparation.**

Std.	Diluent Vol. ( $\mu\text{L}$ )	Standard Vol. Added ( $\mu\text{L}$ )	Survivin Conc. (pg/mL)
1	500	Lyoph.	1,000
2	250	250, Std. 1	500
3	250	250, Std. 2	250
4	250	250, Std. 3	125
5	250	250, Std. 4	62.5
6	250	250, Std. 5	31.25

### • ASSAY PROTOCOL FLOW CHART

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

**HUMAN TOTAL SURVIVIN 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. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

3<sup>rd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

2<sup>nd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

4<sup>th</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_