

human Cytochrome c ASSAY LAYOUT SHEET

for use with Assay Designs' Catalog No. 900-141

• **TABLE FOR MAKING STANDARDS 1-6:**

Reconstitute 450 pg/vial human Cytochrome c standard with 500 μ L of Assay Buffer 13 for a final concentration of 900 pg/mL. Let sit for 5 minutes then mix gently. Subsequent standards should be diluted in polypropylene tubes and used within 60 minutes of preparation. Treat human Cytochrome c standard as a biohazard since it is derived from human placenta.

Std.	Diluent Vol. (μ L)	Standard Vol. Added (μ L)	Cytochrome c Conc. (pg/mL)
1	500	Lyopho.	900
2	250	250, Std. 1	450
3	250	250, Std. 2	225
4	250	250, Std. 3	112.5
5	250	250, Std. 4	56.25
6	250	250, Std. 5	28.125

• **ASSAY PROTOCOL FLOW CHART:**

Well ID:	Blank A1, B1	S0 C1-D1	Standards E1-H2	Samples A3-H12
Standard Diluent	---	100 μ L	---	---
Std. or Sample	---	---	100 μ L	100 μ L
Seal plate and tap gently to mix	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Antibody	---	100 μ L	100 μ L	100 μ L
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	100 μ L	100 μ L	100 μ L
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
pNpp Substrate	100 μ L	100 μ L	100 μ L	100 μ L
Incub. 45 min. @ RT, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution (TSP)	25 μ L	25 μ L	25 μ L	25 μ L
Read OD at 405 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

Cytochrome c 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. _____