

HUMAN TNF- α ASSAY LAYOUT SHEET
for use with Assay Designs' Catalog Number 900-099

• **TABLE FOR MAKING STANDARDS:**

Std.	Diluent Vol. (μ L)	Vol. Added (μ L)	human TNF- α Conc. (pg/mL)
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
7	500	500, Std 6	15.63

• **ASSAY PROTOCOL FLOW CHART:**

Well ID:	Blank A1, B1	S0 C1, D1	Standards E1-B3	Samples C3-H12
Mix all reagents gently	---	---	---	---
Standard Diluent	---	100 μ L	---	---
Std. and/or Sample	---	---	100 μ L	100 μ L
Tap plate gently to mix				
Incub. 2 hours @ 37 °C (sealed)				
Asp. & Wash 4 x 400 μ L				
Antibody	---	100 μ L	100 μ L	100 μ L
Incub. 1 hour @ 37 °C (sealed)				
Asp. & Wash 4 x 400 μ L				
Conjugate	---	100 μ L	100 μ L	100 μ L
Incub. 30 min. @ 37 °C (sealed)				
Asp. & Wash 4 x 400 μ L				
TMB Substrate	100 μ L	100 μ L	100 μ L	100 μ L
Incub. 30 min. @ RT				
Stop Solution 2 (1N HCl)	100 μ L	100 μ L	100 μ L	100 μ L
Read OD at 450 nm				

HUMAN TNF- α PLATE LAYOUT:

A1 Blank	A2 Std 3	A3 Std 7	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 3	B3 Std 7	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. _____