

RAT TNF- α ASSAY LAYOUT SHEET
for use with Assay Designs' Catalog No. 900-086 (formerly 90086)

• **DILUTION TABLE FOR MAKING STANDARDS 1-7:**

* First add 500 μ L of DI water to the vial of Standard, let sit for 5 min., then mix gently.

Std.	Assay Buffer Vol. (μL)	Vol. Added (μL)	rat TNF-α Conc. (pg/mL)
1	220	220, Stock	25,000
2	220	220, Std. 1	12,500
3	220	220, Std. 2	6,250
4	220	220, Std. 3	3,125
5	220	220, Std. 4	1,562.5
6	220	220, Std. 5	781.25
7	220	220, Std. 6	390.63

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	S0 C1, D1	Stds. E1 - B3	Samples C3 - H12
Assay Buffer	---	100 μ L	---	---
Std. and/or Sample	---	---	100 μ L	100 μ L
Tap plate gently & seal				
Incub. 1 hour @ 37°C				
Asp. & Wash 7 x 200 μ L				
Labeled Antibody	---	100 μ L	100 μ L	100 μ L
Seal plate & Incub. 30 min. @ 4°C				
Asp. & Wash 9 x 200 μ L				
Substrate	100 μ L	100 μ L	100 μ L	100 μ L
Incub. 30 min. @ RT, dark				
Stop Solution	100 μ L	100 μ L	100 μ L	100 μ L

• **RAT 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. _____
 2nd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____
 3rd Incub.: Start Time _____ Temp. _____
 End Time _____ Temp. _____

Notes: _____

