

TNF- α (MOUSE) ASSAY LAYOUT SHEET
for use with Enzo Life Sciences Catalog Number ADI-900-047

• **TABLE FOR MAKING STANDARDS:**

Add 120 μ L of deionized water to the vial of 1,200 pg standard, let sit for 5 min., then mix gently. To prepare Std. 1, add 480 μ L of Assay Buffer 16 to this vial and mix gently.

Std.	Diluent Vol. (μ L)	Vol. Added (μ L)	mouse TNF- α Conc. (pg/mL)
1	480	120, Stock (reconstituted in H ₂ O)	2000
2	250	250, Std 1	1000
3	250	250, Std 2	500
4	250	250, Std 3	250
5	250	250, Std 4	125
6	250	250, Std 5	62.5
7	250	250, Std 6	31.25
8	250	250, Std 7	15.63

• **ASSAY PROTOCOL FLOW CHART:**

Well ID:	Blank A1, B1	S0 C1, D1	Standards E1-D3	Samples E3-H12
Mix all reagents gently	---	---	---	---
Std. and/or Sample	---	---	50 μ L	50 μ L
Standard Diluent	---	50 μ L	---	---
Tap plate gently to mix	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Incub. 2 hours @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Antibody	---	50 μ L	50 μ L	50 μ L
Incub. 2 hours @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	50 μ L	50 μ L	50 μ L
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μ L	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
TMB Substrate	50 μ L	50 μ L	50 μ L	50 μ L
Incub. 30 min. @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution 2 (1N HCl)	50 μ L	50 μ L	50 μ L	50 μ L
Read OD at 450nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

TNF- α (MOUSE) 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 Std 8	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 S0	D2 Std 4	D3 Std 8	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. _____