

**NITRIC OXIDE (TOTAL) ASSAY LAYOUT SHEET**  
**for use with Enzo Life Sciences Catalog No. ADI-917-020**

• **DILUTION TABLES FOR MAKING STANDARDS:**

**Nitrate Assay:**

<b>Std.</b>	<b>Reaction Buffer Vol. (µL)</b>	<b>Vol. Added (µL)</b>	<b>Nitrate Conc. (µM)</b>
1	900	100, Stock	100
2	500	500, Std. 1	50
3	500	500, Std. 2	25
4	500	500, Std. 3	12.5
5	500	500, Std. 4	6.25
6	500	500, Std. 5	3.125

• **NITRATE ASSAY PROTOCOL FLOW CHART:**

<b>Well I.D.:</b>	<b>Blank A1, B1</b>	<b>Zero Std. G2, H2</b>	<b>Stds. C1 - F2</b>	<b>Samples A3 - H12</b>
Standard and/or Sample	---	---	50 µL	50 µL
Reaction Buffer	200 µL	50 µL	---	---
Final Diluted NADH	---	25 µL	25 µL	25 µL
Final Diluted Enzyme	---	25 µL	25 µL	25 µL
Mix rgts. by tapping plate				
Incub. 30 min. @ 37 °C, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Griess I	---	50 µL	50 µL	50 µL
Griess II	---	50 µL	50 µL	50 µL
Mix rgts. by tapping plate				
Incub. 10 min. @ RT	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Read @ 550 nm				

• **TOTAL NITRIC OXIDE PLATE LAYOUT:**

A1 Blank	A2 Std 4	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	B2 Std 4	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 Std. 1	C2 Std 5	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 Std. 1	D2 Std 5	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std. 2	E2 Std 6	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std. 2	F2 Std 6	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std. 3	G2 Zero Std.	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std. 3	H2 Zero Std.	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kit Lot No. \_\_\_\_\_ Exp. Date \_\_\_\_\_ Date \_\_\_\_\_ Tech. \_\_\_\_\_

1st Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_ Notes: \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_

2nd Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

End Time \_\_\_\_\_ Temp. \_\_\_\_\_