

**24(S)-Hydroxycholesterol ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences Catalog No. ADI-900-210

• **TABLE FOR MAKING STANDARDS 1-9:**

Diluted standards should be used immediately.

Std	Assay Buffer Vol. (μL)	Standard Vol. Added (μL)	24(S)-Hydroxycholesterol Conc. (ng/mL)
1	990	10, 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
7	500	500, Std. 6	1.56
8	500	500, Std. 7	0.78
9	500	500, Std. 8	0.39

• **ASSAY PROTOCOL FLOW CHART:**

Well I.D.:	Blank A1, B1	NSB C1, D1	Bo G3, H3	Standards E1-F3	Samples A4-H12
Assay Buffer	---	150 μL	100 μL	---	---
Std. and/or Sample	---	---	---	100 μL	100 μL
Conjugate	---	50 μL	50 μL	50 μL	50 μL
Antibody	---	---	50 μL	50 μL	50 μL
Incub. 1 hrs. @ RT, shaking (sealed)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
SA-HRP	---	200 μL	200 μL	200 μL	200 μL
Incub. 30 min. @ RT, shaking (sealed)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x 400 μL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
TMB Substrate	200 μL	200 μL	200 μL	200 μL	200 μL
Incub. 30min @ RT <b>without shaking</b>	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 μL	50 μL	50 μL	50 μL	50 μL
Read @ 450 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

24(S)-Hydroxycholesterol 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 NSB	C2 Std 4	C3 Std 8	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 NSB	D2 Std 4	D3 Std 8	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std 1	E2 Std 5	E3 Std 9	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std 1	F2 Std 5	F3 Std 9	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std 2	G2 Std 6	G3 Bo	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 2	H2 Std 6	H3 Bo	H4	H5	H6	H7	H8	H9	H10	H11	H12

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

1<sup>st</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

2<sup>nd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

3<sup>rd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_