13(S)-HODE ASSAY LAYOUT SHEET

for use with Enzo Life Sciences Catalog No. ADI-900-108

• DILUTION TABLE FOR MAKING STANDARDS 1-5:

Std.	Diluent Vol. (µL)	Vol. Added (µL)	13(S)-HODE Conc. (ng/mL)
1	900	100, Stock	1,000
2	750	250, Std. 1	250
3	750	250, Std. 2	62.5
4	750	250, Std. 3	15.6
5	750	250, Std. 4	3.9

• ASSAY PROTOCOL FLOW CHART:

Well I.D.:	Blank A1, B1	TA C1, D1	NSB E1, F1	Bo G1, H1	Stds. A2 - B3	Samples C3 - H12
Assay Buffer	---	---	50 µL	---	---	---
Standard Diluent	---	---	100 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. 2 hours RT, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 3 x 400 µL	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Conjugate	---	**5 µL***	---	---	---	---
Substrate	200 µL	200 µL	200 µL	200 µL	200 µL	200 µL
Seal plate & Incub. 2 hours at 37 °C	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution	50 µL	50 µL	50 µL	50 µL	50 µL	50 µL

Conjugate for TA must be diluted 1:10 in standard diluent. Add 500 µL of standard diluent to a clean test tube. From this volume, remove 50 µL of standard diluent and, using the same measuring device, add 50 µL of 13(S)-HODE Conjugate.

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• 13(S)-HODE PLATE LAYOUT:

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

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