

Insulin ELISA ASSAY LAYOUT SHEET
for use with Enzo Life Sciences' Catalog No. ENZ-KIT141

• **TABLE FOR MAKING STANDARDS 1-6:**

Std.	AB13 Vol. (µL)	Standard Vol. Added (µL)	Insulin Conc. (pg/mL)
1	990	10, stock	500
2	500	500, Std. 1	250
3	500	500, Std. 2	125
4	500	500, Std. 3	62.5
5	500	500, Std. 4	31.3
6	500	500, Std. 5	15.6

• **ASSAY PROTOCOL FLOW CHART: Vol. Added (µL)**

Well ID:	TA	NSB	S0	Standards	Samples
	A1, B1	C1, D1	E1-F1	G1-B3	C3-H12
Mix all reagents gently	---	---	---	---	---
Assay Buffer 13	---	100 µL	100 µL	---	---
Std. and/or Sample	---	---	---	100 µL	100 µL
Incub. 60 min @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full-well volume (~400 µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Assay Buffer 13	---	100 µL	---	---	---
Insulin Antibody	---	---	100 µL	100 µL	100 µL
Incub. 60 min @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full-well volume (~400 µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Insulin Conjugate	---	100 µL	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full-well volume (~400 µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
TA (1:100 dilution of Conjugate in Assay Buffer 13)	5 µL	---	---	---	---
TMB Substrate	100 µL	100 µL	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, sealed, shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution 2 (1N HCL)	100 µL	100 µL	100 µL	100 µL	100 µL
Read OD at 450 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

INSULIN PLATE LAYOUT:

A1 TA	A2 Std 2	A3 Std 6	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 TA	B2 Std 2	B3 Std 6	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1 NSB	C2 Std 3	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 NSB	D2 Std 3	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1 Std 0	E2 Std 4	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Std 0	F2 Std 4	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1 Std 1	G2 Std 5	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 Std 1	H2 Std 5	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. _____