

Transferrin ELISA ASSAY LAYOUT SHEET
for use with Enzo Life Sciences' Catalog No. ENZ-KIT143

• **TABLE FOR MAKING STANDARDS 1-6:**

Std.	AB13 Vol. (µl)	Standard Vol. Added (µl)	Transferrin Conc. (ng/ml)
1	450	50, stock	5000
2	375	125, Std. 1	1250
3	375	125, Std. 2	312.5
4	375	125, Std. 3	78.1
5	375	125, Std. 4	19.5
6	375	125, Std. 5	4.9

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

	Blank	NSB	Bo	Standards	Samples
Well ID:	A1, B1	C1-D1	E1-F1	G1-B3	C3-H12
Assay Buffer 13	---	150µl	100µl	---	---
Standards or Samples	---	---	---	100µl	100µl
Transferrin Tracer	---	50µl	50µl	50µl	50µl
Transferrin Antibody	---	---	50µl	50µl	50µl
Incub. 1 hour @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full well vol. (~400µl)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
SA-HRP Conjugate	---	200µl	200µl	200µl	200µl
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full well vol. (~400µl)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
TMB Substrate	200µl	200µl	200µl	200µl	200µl
Incub. 30 min. @ RT, shaking, sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution 2 (1N HCl)	50µl	50µl	50µl	50µl	50µl
Read OD at 450 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

Transferrin PLATE LAYOUT:

A1 Blank	A2 Std 2	A3 Std 6	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1 Blank	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 Bo	E2 Std 4	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1 Bo	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. _____

End Time _____ Temp. _____

2nd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____

3rd Incub.: Start Time _____ Temp. _____

End Time _____ Temp. _____