

**VEGF (human) ELISA ASSAY LAYOUT SHEET**  
for use with Enzo Life Sciences' Catalog No. ENZ-KIT156-0001

• **TABLE FOR MAKING STANDARDS 1-6:**

Std.	AB10 Vol. (µL)	Standard Vol. Added (µL)	VEGF Conc. (pg/mL)
1	580	20, stock	2000
2	300	150, Std. 1	667
3	300	150, Std. 2	222
4	300	150, Std. 3	74
5	300	150, Std. 4	25
6	300	150, Std. 5	8

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

Well ID:	Blank A1, B1	TA C1, D1	NSB E1, F1	S0 G1-H1	Standards A2-D3	Samples E3-H12
Assay Buffer 10	---	---	100 µL	100 µL	---	---
Standards or Samples	---	---	---	---	100 µL	100 µL
Incub. 60 min. @ RT, sealed & shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full well vol. (~400µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Assay Buffer 10	---	---	100 µL	---	---	---
Biotinylated VEGF 1X Detector Antibody	---	---	---	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, sealed & shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full well vol. (~400µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
VEGF Conjugate	---	---	100 µL	100 µL	100 µL	100 µL
Incub. 30 min. @ RT, sealed & shaking	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Asp. & Wash 4 x Full well vol. (~400µL)	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Total Activity (TA)		*5 µL				
TMB Substrate	100 µL	100 µL	100 µL	100 µL	100µL	100 µL
Incub. 30 min. @ RT, shaking & sealed	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒
Stop Solution 2 (1N HCl)	100 µL	100 µL	100 µL	100 µL	100 µL	100 µL
Read OD at 450 nm	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒	⇒⇒⇒⇒

\*Conjugate for TA must be diluted 1:50 in Assay Buffer 10.

**VEGF 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 Std 6	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1 TA	D2 Std 2	D3 Std 6	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 S0	G2 Std 4	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1 S0	H2 Std 4	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

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

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

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

2<sup>nd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_ 4<sup>th</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_

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