



Protein Standards for Human Angiogenesis I ELISA Strip

Catalog Number EA-1012

(For Research Use Only)

Protein Standards

<u>Tube</u>	<u>Protein</u>	<u>Stock Conc.</u>	<u>Volume</u>
#1	Human TNFa	400ng/ml	10ul
#2	Human IGF-1	400ng/ml	10ul
#3	Human VEGF	400ng/ml	10ul
#4	Human IL-6	400ng/ml	10ul
#5	Human FGFb	400ng/ml	10ul
#6	Human TGFb	400ng/ml	10ul
#7	Human EGF	400ng/ml	10ul
#8	Human Leptin	400ng/ml	10ul

Preparation of protein standard dilutions

1. Add 200ul of Diluent buffer the wells of the first strip, and add 100ul of Diluent buffer to the wells of the rest strips according to the following table.
2. Add appropriate amount of protein standards to the first strip according to the following table.
3. Use multi-channel pipette to mix the dilutions and transfer 100ul to the next dilution wells.
4. Repeat the transfer until 5th strip. Discard 100ul from 5th strip after mixing. Do not transfer to the 6th Strip

Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes. Always stop protein standards along with samples from the same row at the same time.

Table 1: Dilution of protein standards

<u>Protein</u>	<u>Added to 1st Strip</u>	<u>1st Strip</u>	<u>2nd Strip</u>	<u>3rd Strip</u>	<u>4th Strip</u>	<u>5th Strip</u>	<u>6th Strip</u>
Human TNFa	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human IGF-1	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human VEGF	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human IL-6	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human FGFb	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human TGFb	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human EGF	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Human Leptin	4ul	200ul	100ul	100ul	100ul	100ul	100ul
			1:2	1:4	1:8	1:16	Blank

Table 2: Concentrations of Protein Standard Dilutions

<u>Protein</u>		<u>1:2</u>	<u>1:4</u>	<u>1:8</u>	<u>1:16</u>	<u>Blank</u>
Human TNFa	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human IGF-1	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human VEGF	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human IL-6	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human FGFb	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human TGFb	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human EGF	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Human Leptin	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank