

## Protein Standards for Rat Angiogenesis ELISA Strip

Catalog Number EA-1212

(For Research Use Only)

## **Protein Standards**

<u>Tube</u>	<u>Protein</u>	Stock Conc.	<u>Volume</u>
#1	Rat TNFa	400ng/ml	10ul
#2	Rat VEGF	400ng/ml	10ul
#3	Rat IL-6	400ng/ml	10ul
#4	Rat FGFb	400ng/ml	10ul
#5	Rat IFNr	400ng/ml	10ul
#6	Rat Leptin	400ng/ml	10ul
#7	Rat MCP-1	400ng/ml	10ul
#8	Rat Rantes	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 1ul of diluted 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 5<sup>th</sup> strip. Trash 100ul from 5<sup>th</sup> strip after mixing. Do not transfer to the 6<sup>th</sup> 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

						100ul		
		100	ul 100	ul 100	Oul 10	00ul	V	
		D				D =	/	
	Added to 1st							
<u>Protein</u>	<u>Strip</u>	1st Strip	2nd Strip	3rd Strip	4th Strip	5th Strip	6th Strip	
Rat TNFa	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat VEGF	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat IL-6	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat FGFb	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat IFNr	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat Leptin	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat MCP-1	4ul	200ul	100ul	100ul	100ul	100ul	100ul	
Rat Rantes	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>
Rat TNFa	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat VEGF	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat IL-6	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat FGFb	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat IFNr	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat Leptin	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat MCP-1	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat Rantes	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank