



## Rat Oxidative Stress ELISA Strip Protein Standards

Catalog Number EA-1502

(For Research Use Only)

<u>Tube</u>	<u>Protein</u>	<u>Stock Conc.</u>	<u>Volume</u>
#1	Rat TNF $\alpha$	400ng/ml	10ul
#2	Rat TGF $\beta$	400ng/ml	10ul
#3	Rat MCP-1	400ng/ml	10ul
#4	Rat IL-1 $\alpha$	400ng/ml	10ul
#5	Rat IL-1 $\beta$	400ng/ml	10ul
#6	Rat IL-6	400ng/ml	10ul
#7	Rat IL-15	400ng/ml	10ul
#8	Rat VEGF	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 of the strips according to the following table.
2. Add appropriate amount of protein standards to the first strip according to the table 1.
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**

<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>
Rat TNF $\alpha$	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat TGF $\beta$	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat MCP-1	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat IL-1 $\alpha$	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat IL-1 $\beta$	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat IL-6	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat IL-15	4ul	200ul	100ul	100ul	100ul	100ul	100ul
Rat VEGF	4ul	200ul	100ul	100ul	100ul	100ul	100ul
			<b>1:2</b>	<b>1:4</b>	<b>1:8</b>	<b>1:16</b>	<b>Blank</b>

**Table 2: Concentrations of Protein Standard Dilutions**

<b><u>Protein</u></b>		<b><u>1:2</u></b>	<b><u>1:4</u></b>	<b><u>1:8</u></b>	<b><u>1:16</u></b>	<b><u>Blank</u></b>
Rat TNF $\alpha$	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat TGF $\beta$	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 IL-1 $\alpha$	8ng/ml	4ng/ml	2ng/ml	1ng/ml	0.5ng/ml	Blank
Rat IL-1 $\beta$	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 IL-15	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