



## Rat Angiogenesis ELISA Strip for Profiling 8 Cytokines

Catalog Number EA-1211

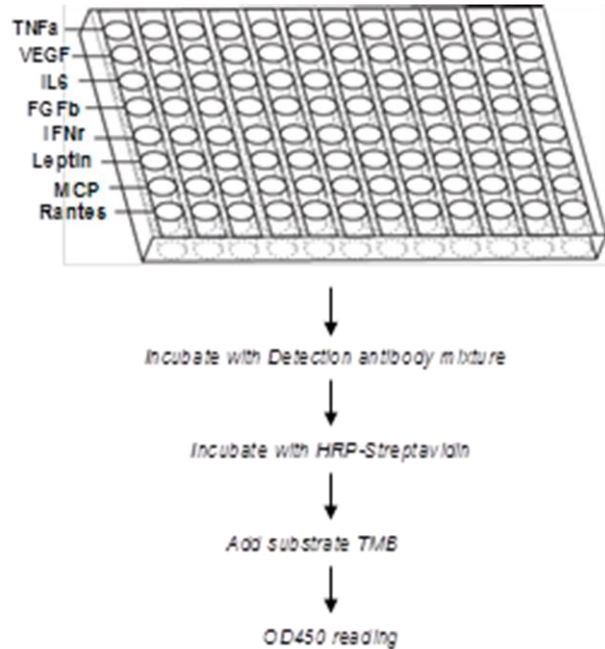
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### Introduction

Angiogenesis shifted from the avascular to vascular state is a key event for sustained tumor growth and cancer progression. Angiogenesis as a biological switch process is governed by numerous pro- and anti-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGFb) and IL-6. The mechanism of action of each of these factors is different, as are their origin and the stimuli for their production. The angiogenic switch refers to the balance between pro- and anti- angiogenic factors. Therefore, profiling of these factors is critical to understanding angiogenesis. Signosis's Rat Angiogenesis ELISA Strip Profiling Assay simultaneously profile 8 rat angiogenesis cytokines; TNF $\alpha$ , VEGF, IL-6, FGFb, IFN $\gamma$ , Leptin, MCP-1 and Rantes. The difference of these proteins between two samples can be determined through data comparison.

### Principle of the assay

In each well of the strip, a primary antibody against a specific angiogenesis cytokine is coated and 8 wells of the strip are coated with 8 different antibodies. Therefore, total 8 wells of a strip allow measurement of 8 different cytokines. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the angiogenesis cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentrations of the angiogenesis cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



### Materials provided with the kit

Component	Qty	Store at
<b>96-Well 12 strip Plate coated with 8 different antibodies against rat angiogenesis cytokines</b>	1	4°C
<b>Biotin-labeled antibody mixture against 8 different rat angiogenesis cytokines</b>	200 $\mu$ L	-20°C
<b>Streptavidin-HRP conjugate</b>	50 $\mu$ L	4°C
<b>1xDiluent buffer</b>	40mL	4°C
<b>5X Assay wash buffer</b>	40mL	4°C
<b>Substrate</b>	10mL	4°C
<b>Stop solution</b>	5mL	RT

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer:
  - 40ml 5x Assay wash buffer
  - 160ml ddH<sub>2</sub>O
- To dilute standards, refer to Standards User Manual.
- Dilute biotin-labeled antibody mixture 1:50 with 1X Diluent Buffer.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer.

## Sample preparation before starting experiment

- For **cell culture medium samples**, add 100µl directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer, for example, add 80ul sample in 720ul 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control

## Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the angiogenesis cytokines among the samples can be easily identified and determined.
- If you would like to quantitatively measure the cytokines in the samples, please order EA-1212. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Following EA-1212 user manual)

## Assay procedure

1. Take the desired number of well strips from the plate.  
Make sure the rest of strips are well sealed.
  2. Standard curve:  
If protein standard curve is desired, 4-5 strips may be used to make Standard curve (Please see the user manual for EA-1212 for detail).
  3. Sample assay:  
Apply each sample in one strip, 100ul per well and incubate for 1-2 hour at room temperature with gentle shaking.
  4. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
  5. Add 100µl of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
  6. Repeat the aspiration/wash as in step 4.
  7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
  8. Repeat the aspiration/wash as in step 4.
  9. Add 100µl substrate to each well and incubate for 10-30 minutes.
- 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 the reaction of samples from the same row at the same time.**
10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
  11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

## Rat Angiogenesis ELISA Strip Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa	TNFa
B	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF
C	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
D	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb	FGFb
E	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr	IFNr
F	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin	Leptin
G	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
H	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes	Rantes