Signosis
Innovative Plate Assay Solutions

Human Angiogenesis ELISA Strip II (Colorimetric)

Catalog Number EA-1041 **(For Research Use Only)**

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), epidermal growth factor (EGF), and transforming growth factor-beta (TGF-b). 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' Angiogenesis ELISA Strip Profiling Assay simultaneously profiles 8 angiogenesis cytokines; PDGF-BB, PIGF-1, NGF, SCF, MCP-1, MIP-1a, IL-2, and IL-4. 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.

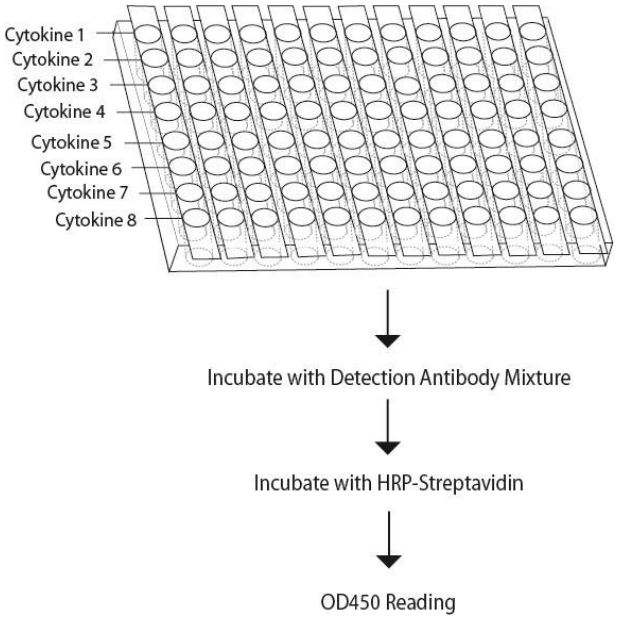

Diagram of Human Angiogenesis ELISA Strip II

Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with 8 different antibodies against human angiogenesis cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human angiogenesis cytokines	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1x Diluent buffer	40 mL	4°C
5x Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O .
- Dilute 50 times of biotin labeled antibody mixture with 1x Diluent buffer.
- Dilute 200 times of streptavidin-HRP 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 80µl sample in 720µl 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 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-1042. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions. (Follow EA-1042 user manual)
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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-1042 for detail).
3. Sample assay:
Apply each sample in one strip, 100µl 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 100µl 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.

Human Angiogenesis ELISA Strip II Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB	PDGF-BB
B	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1	PIGF-1
C	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF	β-NGF
D	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF	SCF
E	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
F	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α	MIP-1α
G	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2	IL-2
H	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4	IL-4