Signosis
BioSignal Capture

Human Angiogenesis ELISA Strip I for Profiling 8 Cytokines

Catalog Number EA-1011 **(For Research Use Only)**

Introduction

Angiogenesis shifted from the avascular to vascular states is a key event for sustained tumor growth and cancer progression (1). 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 allows simultaneously profiling 8 angiogenesis cytokines; TNFa, IGF-1, VEGF, IL-6, FGFb, TGFb, EGF, and Leptin. Each well of the strip is coated with a primary antibody against a specific angiogenesis protein and total 8 wells of a strip target 8 different proteins. 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.

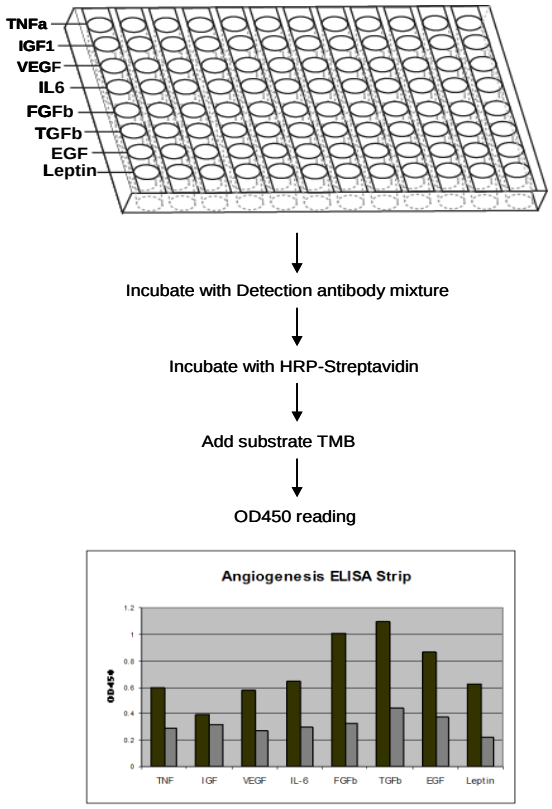


Diagram of Human Angiogenesis ELISA Strip I

Materials provided with the kit

- 12 strips, each coated with 8 different antibodies against angiogenesis cytokines (4°C).
- Biotin labeled antibody mixture I against 8 different angiogenesis cytokines (-20°C).
- Streptavidin-HRP conjugate (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (RT)
- Substrate (4°C)
- Stop Solution (4°C)

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 50 times of biotin labeled antibody mixture I with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

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-1012. It is protein standards which can be used for making standard curves through a series of 2-fold dilutions.

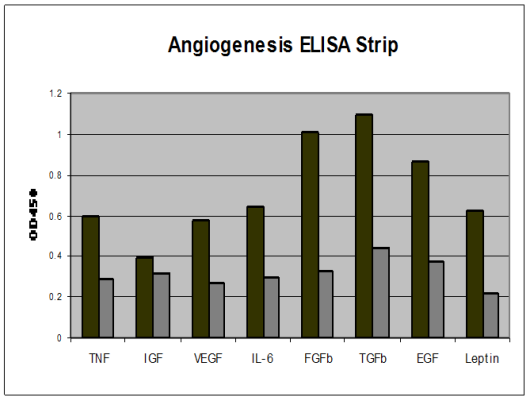
Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Add 100 µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100µl of diluted biotin-labeled antibody mixture I to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl substrate to each well and incubate for 10-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

(1) Folkman, J. 1971. Tumour angiogenesis: therapeutic implications. N. Engl. J. Med. 285, 1182–1186.

Example of standard curve



	Black bar	Grey bar
TNFα	290pg/ml	72.5pg/ml
IGF1	220pg/ml	55pg/ml
VEGF	600pg/ml	150pg/ml
IL6	35pg/ml	6.25pg/ml
FGFb	800pg/ml	200pg/ml
TGFβ	280pg/ml	70pg/ml
EGF	1ng/ml	0.25ng/ml
Leptin	5ng/ml	1.25ng/ml