Signosis
BioSignal Capture

Human Growth Factor ELISA Strip II for Profiling 8 Proteins

Catalog Number EA-1101 **(For Research Use Only)**

Introduction

Growth factors are a family of proteins capable of stimulating cellular growth, proliferation and cellular differentiation. They typically act as signaling molecules by binding to receptors on the cell surface. Many growth factors are quite versatile, stimulating cellular division in numerous different cell types; while others are specific to a particular cell-type. Multiple growth factors often together participate in cellular functions. For example, VEGF, FGF, and EGF stimulate angiogenesis. Therefore, profiling the expression pattern of growth factors provides a valuable insight to the biological mechanisms underlying cellular functions. Signosis' Growth Factor ELISA Strip II allows quantitatively profiling and measuring 8 proteins; VEGF, EGF, PDGF-BB, NGF-b, SCF, TNFa, FGFb, and TGFb.

Principle of the assay

In each well of the strip, a primary antibody against a specific growth factor 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 growth factors. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the growth factors 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 growth factors are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

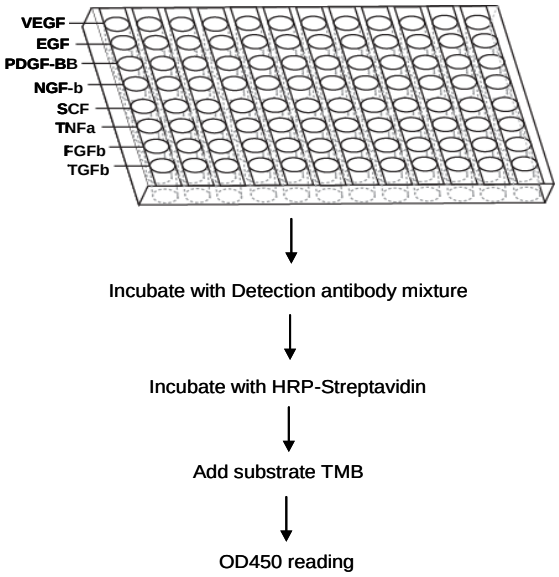


Diagram of Human Growth Factor ELISA Strip II

Materials provided with the kit

- 12 strips, each coated with 8 different antibodies against human growth factors (4°C).
- Biotin labeled antibody mixture against 8 different human growth factors (-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 with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

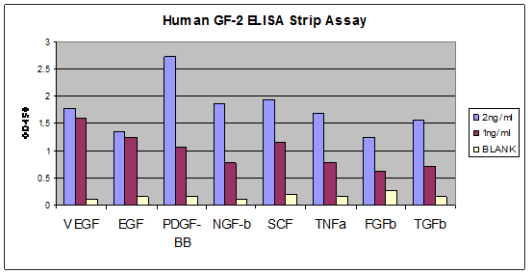
Recommendation

- The product intends to be used for comparison of 12 different samples. The differences of the growth factors among the samples can be easily identified and determined.
- If you would like to quantitatively measure the growth factors in the samples, please order EA-1102. 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 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 5-30 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.

Example of standard curve



	Blue	Red	Yellow
VEGF	2ng/ml	1ng/ml	Blank
EGF	2ng/ml	1ng/ml	Blank
PDGF-BB	2ng/ml	1ng/ml	Blank
NGF-b	2ng/ml	1ng/ml	Blank
SCF	2ng/ml	1ng/ml	Blank
TNFa	2ng/ml	1ng/ml	Blank
FGFb	2ng/ml	1ng/ml	Blank
TGFb	2ng/ml	1ng/ml	Blank