Reagent for research use　MK ELISA kit

Midkine (MK) is a basic heparin-binding growth factor of low molecular weight, and forms a family with pleiotrophin (NEGF1, 46% homologous with MK). It is a nonglycosylated protein, composed of two domains held by disulfide bridges. It is a developmentally important retinoic acid-responsive gene product strongly induced during mid-gestation, hence the name midkine. Restricted mainly to certain tissues in the normal adult, it is strongly induced during oncogenesis, inflammation and tissue repair.

【Contents of the kit】

1) Anti-MK monoclonal antibody immobilized on 96-well Plate (1plate)
2) Peroxidase (POD)-labeled anti-THP monoclonal antibody (150μL×1)
3) Synthesized THP standard (8ng/tube×1)
4) Sample diluent buffer (50mL×1)
5) 10-time concentrated washing buffer (50 mL×1)
6) Chromogenic substrate: 3, 3', 5, 5'-tetramethyl benzidine (TMB)　(12mL×1)
7) Stop solution: sulfuric acid (12mL×1)
*Kit should be stored at 2-8℃

【Sample】

Human serum and Plasma

【Operating procedure】

All of reagents are used at room temperature.

1) Preparation of washing buffer
Make a 10-time dilution of washing buffer in purified water.

2) A series of 2-time diluted standards
Prepare 8000pg/mL of MK solution by adding 1 mL of sample diluent buffer to MK standard, and then make a series of 2-time THP standard dilutions (4,000, 2,000, 1,000, 500, 250, 125, 62.5, 0 pg/mL (only diluted buffer).

3) Sample preparation

Make a 2-time diluted human serum (or Plasma) in sample diluent buffer. In case of high concentration of sample, sample is diluted appropriately.

4) Preparation of POD-labeled anti-THP monoclonal antibody

Use 100-time diluted POD-labeled antibody by adding 120 μL of POD-labeled antibody to 12 mL of sample diluent buffer.

【Method of measurement】

Add 100 μL of a series of prepared standards and diluted samples to each well of 96-well plate.

↓ allow to stand at RT for 1 hour

Wash each well 4 times in washing buffer (300 μL/well).

After that, add 100 μL of POD-labeled antibody solution to each well.

↓ allow to stand at RT for 1 hour

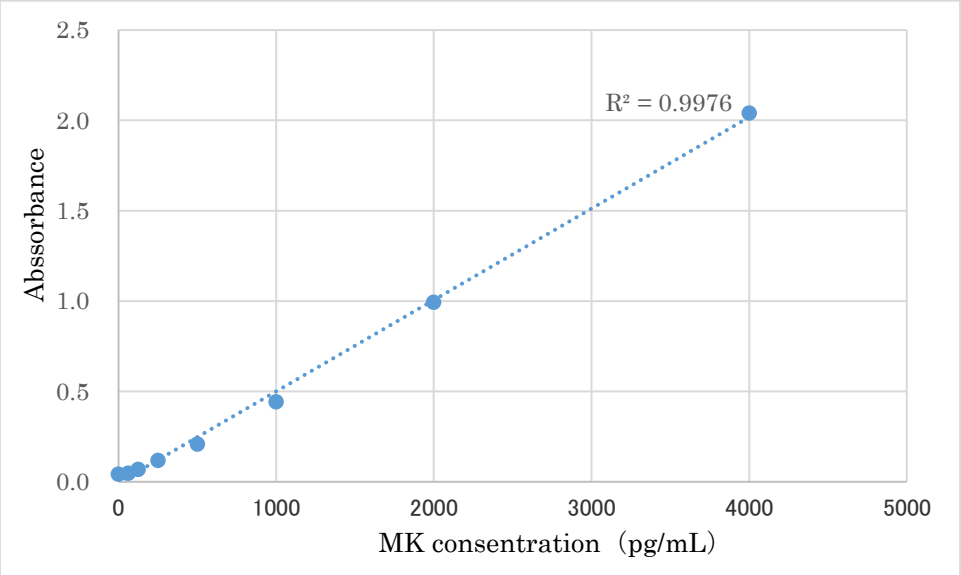
Wash each well 4 times in washing buffer (300μL/well).

Add 100 μL of Chromogenic substrate to each well.

↓ block light and allow to stand at RT for half hour

After adding 100 μL of stop solution to each well, measure each well at 450 nm / 650 nm (main wavelength/ sub wavelength)

Representative calibration curve



【Storage】

2 ～ 8 ℃