Reagent for research use　THP ELISA kit

Tamm-Horsfall protein (uromodulin) is the most abundant glycoprotein in human urine, which is synthesized exclusively in the thick ascending limb of the loop of Henle. It has recently been demonstrated that familial juvenile hyperuricemic nephropathy and medullary cystic kidney disease are caused by THP mutations. Some researchers have developed a non-invasive quantitative assay for urinary THP as a potential biomarker for the diagnosis of a kidney stone disease. Our established ELISA shows a linearity within a range of 1.25 ng/ml to 80 ng/ml and a sensitivity of 0.78 ng/ml.

【Contents of the kit】

1) Anti-THP monoclonal antibody immobilized on 96-well Plate (1plate)
2) Peroxidase (POD)-labeled anti-THP monoclonal antibody (375 μ L×1)
3) Freeze-dried THP standard (40ng/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

【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 40 ng/mL of THP solution by adding 1 mL of sample diluent buffer to THP standard, and then make a series of 2-time THP standard dilutions (20, 10, 5, 2.5, 1.2, 0.6, 0.3, 0 ng/mL (only diluted buffer).

3) Sample preparation
Dilute human serum in sample diluent buffer from 10 times to 50 times.

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

Use 40-time diluted POD-labeled antibody by adding 350 μL of POD-labeled antibody to 14 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

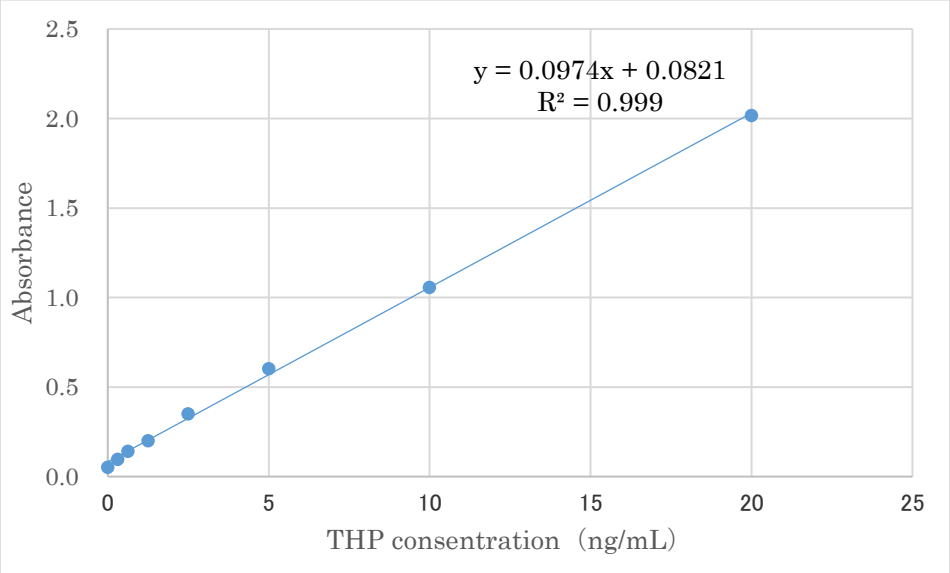
Wash each well 4 times in washing buffer (300μL/well).

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 ℃