

StepSkip™ Porcine MP ELISA Kit

Cat# E5018

Storage at 2-8°C for 6 month

INTRODUCTION

This kit allows for the determination of MP concentrations in Porcine serum, plasma and other biological fluids.

SPECIFICATION

Detection Range: 6-160 pg/mL

PRINCIPLE of KIT

The kit assay Porcine MP level in the sample, use Purified Porcine MP antibody to coat microtiter plate wells, make solid-phase antibody, then add MP to wells, Combined MP antibody which With HRP labeled, become antibody - antigen - enzyme-antibody complex, after washing Completely, Add TMB substrate solution, TMB substrate becomes blue color At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of Porcine MP in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials provided with the kit

1	washsolution	20ml×1bottle	7	Stop Solution	6ml×1 bottle
2	HRP-Conjugate reagent	6ml×1 bottle	8	Standard (320pg/ml)	0.5ml×1 bottle
3	Microelisa stripplate	12well×8strips	9	Standard diluent	1.5ml×1bottle
4	Sample diluent	6ml×1 bottle	10	Instruction	1
5	Chromogen Solution A	6ml×1 bottle	11	Closure plate membrane	2
6	Chromogen Solution B	6ml×1 bottle	12	Sealed bags	1

Specimen requirements

1. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20°C to preserve, Avoid repeated freeze-thaw cycles.
2. Can't detect the sample which contain NaN_3 , because NaN_3 inhibits HRP active.

STORAGE

An unopened kit can be stored at $2-8^{\circ}\text{C}$ for six months.

NOTE

1. The kit takes out from the refrigeration environment should be balanced 15-30 minutes in the room temperature, ELISA plates coated if has not use up after opened, the plate should be stored in Sealed bag.
2. washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute . Washing does not affect the result.
3. add Sample with sampler Each step, And proofread its accuracy frequently, avoids the experimental error. add sample within 5 min, if the number of sample is much , recommend to use Volley .
4. if the testing material content is excessively higher (The sample OD is bigger than the first standard well), please dilute Sample (n-fold), Please diluente and multiplied by the dilution factor. ($\times n \times 5$) .
5. Closure plate membrane only limits the disposable use, to avoid cross-contamination.
6. The substrate evade the light preservation.
7. Please according to use instruction strictly, The test result determination must take the microtiter plate reader as a standard.
8. All samples, washing buffer and each kind of reject should according to infective material process.
9. Do not mix reagents with those from other lots.

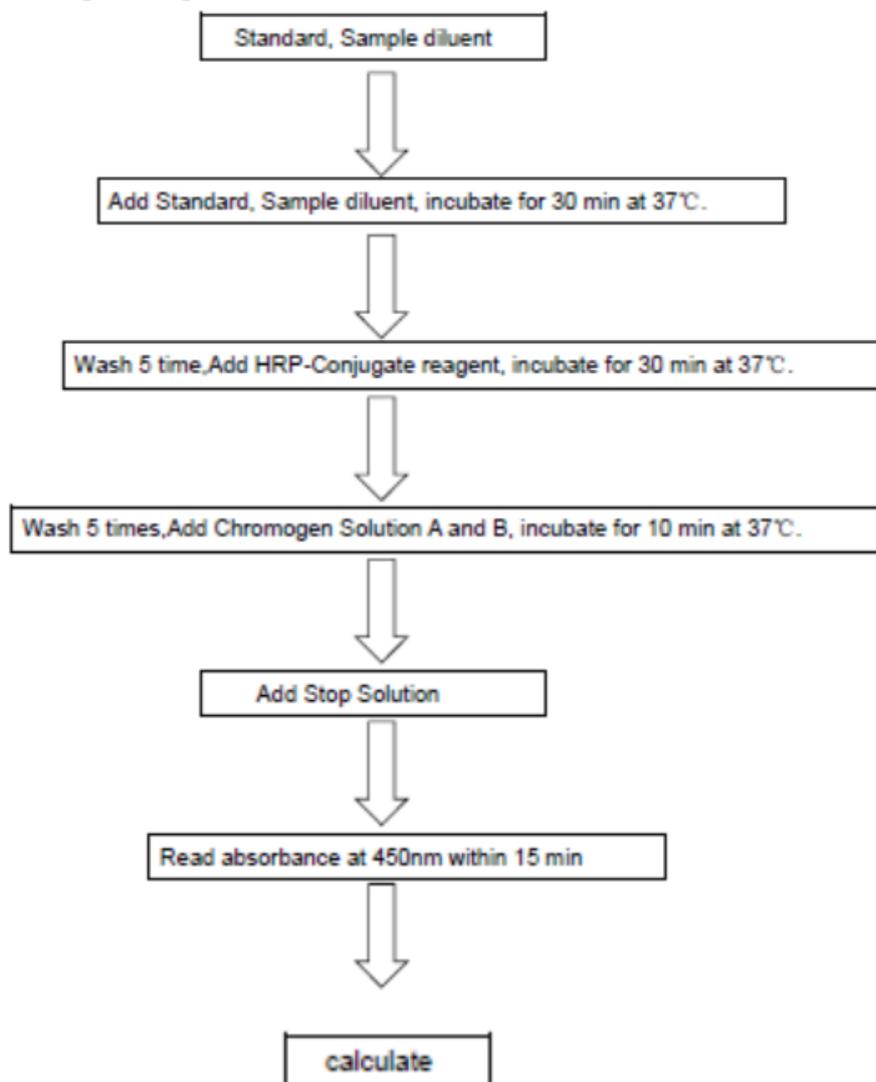
ASSAY PROCEDURE

1. Dilute and add sample: Dilute Original density Standard as follow table:

160pg/ml	5 Standard	150µl Original density Standard+150µl Standard diluent
80pg/ml	4 Standard	150µl 5 Standard+150µl Standard diluent
40pg/ml	3 Standard	150µl 4 Standard+150µl Standard diluent
20pg/ml	2 Standard	150µl 3 Standard +150µl Standard diluent
10pg/ml	1 Standard	150µl 2 Standard +150µl Standard diluent

2. Add sample: Set blank wells separately (blank comparison wells don't add sample and HRP-Conjugate reagent, other each step operation is same). testing sample well. Add 50µl of standard to Microelisa stripplate, add Sample dilution 40µl to testing sample well, then add testing sample 10µl (sample final dilution is 5-fold), add sample to wells, don't touch the well wall as far as possible, and Gently mix.
3. Incubate: After closing plate with Closure plate membrane, incubate for 30 min at 37°C.
4. Configurated liquid: 30-fold (or 20-fold) wash solution diluted 30-fold (or 20-fold) with distilled water and reserve.
5. Washing: Uncover Closure plate membrane, discard Liquid, dry by swing, add washing buffer to every well, still for 30s then drain, repeat 5 times, dry by pat.
6. Add enzyme: Add HRP-Conjugate reagent 50µl to each well, except blank well.
7. Incubate: Operation with 3.
8. Washing: Operation with 5.
9. Color: Add 50µl Chromogen Solution A and 50µl Chromogen Solution B to each well, evade the light preservation for 10 min at 37°C
10. Stop the reaction: Add Stop Solution 50µl to each well, Stop the reaction (the blue color change to yellow color).
11. Assay: take blank well as zero, Read absorbance at 450nm after Adding Stop Solution and within 15min.

Steps description



CALCULATION of RESULTS

The actual concentration is the calculated concentration multiplied by the dilution factor. Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.

PRODUCT USE LIMITATION

These products are intended for research use only.