Assay range: 25 ng/L-600ng/L **96determinations**

Purpose

This kit allows for the determination of B12 concentrations in Human serum, cell culture supernates and other biological fluids

Principle of the assay

The kit assay Human VB12 level in the sample, use Purified Human VB12 antibody to coat microtiter plate wells, make solid-phase antibody, then add VB12 to wells, Combined VB12 antibody which With HRP labeled goat anti-Human 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 VB12 in the samples is then determined by comparing the O.D. of the samples to the standardcurve.

Materials provided with the kit

1	wash solution	20ml×1bottle	7	Stopp Solution	6ml×1 bottle
2	HRP-Conjugate reagent	6ml×1 bottle	8	Standard (1200ng/L)	0.5ml×1 bottle
3	Microelisastripplate	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

- 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 ℃ to preserve, Avoid repeated freeze-thawcycles.
- Can't detect the sample which contain NaN3, because NaN3 inhibits HRPactive.

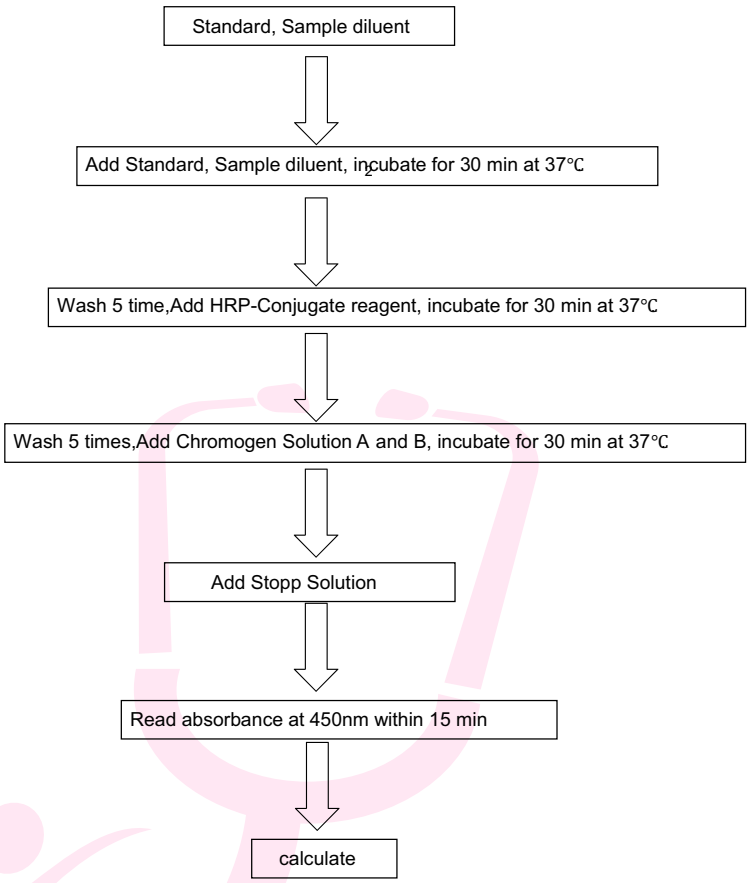
Assay procedure

- Dilute and add sample:DiluteOriginal density Standard as followtable:

600ng/L	5 Standard	150μl Original density Standard+150μl Standard diluent
300ng/L	4 Standard	150μl 5 Standard+150μl Standard diluent
150ng/L	3 Standard	150μl 4Standard+150μl Standard diluent
75ng/L	2 Standard	150μl 3Standard +150μl Standard diluent
37.5 ng/L	1 Standard	150μl 2Standard +150μl Standard diluent

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 Sample dilution40μltotestingsamplewell,thenaddtestingsample10μl(samplefinaldilutionis 5-fold), addsampletowells,don'ttouchthewellwallasfaraspossible,andGentlymix. Incubate: After closing plate with Closure plate membrane ,incubate for 30 min at 37℃. 4.Configurateliquid:30-fold(or20-fold)washsolutiondiluted30-fold(or20-fold)withdistilled water and reserve.

Steps description



Calculate

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 actualdensity.

Important notes

- 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 Sealedbag.
- washing buffer will Crystallization separation, it can be heated the water helps dissolve when dilute . Washing does not affect theresult.
- 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 .
- 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. (×n×5)

Vitamin B12 ELISA