**Human Citrulline (Citrulline)ELISA Kit (for ALCHEMY plasma)**

**Product Specifics**

Catalog No: EK713215 For the quantitative determination of Human Citrulline concentrations.

Product Principle: The kit is for the quantitative level of Human Citrulline in the sample, adopt purified Citrulline antibody to coat microtiter plate, make solid-phase antibody, then add Citrulline to wells, Combine Citrulline antibody with labeled HRP to form 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 stop solution and the color change is measured at a wavelength of 450 nm. The concentration of Citrulline in the samples is then determined by comparing the O.D. of the samples to the standard curve.

**Specimen requirements**

Plasma: use suited EDTA or citrate plasma as an anticoagulant, centrifuge at the speed of 2000-3000 rpm for 20-min. Remove supernatant, if precipitation appeared, centrifuge again. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

**Procedure**

Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (30x) into deionized or distilled water to prepare 600ml of Wash Buffer (1x).

Standard Dilution: Pipette 50μl standard diluent in each tube. Pipette 100μl standard (135 nmol/ml) in the fifth tube. Take out 100μl from the fifth five tube into the fourth. Pipette 50μl from the fourth tube to the third tube, 50μl from the third to the second, 50μl from the second to the first. The undiluted Standard serves as the high standard (135 nmol/ml). Sample Diluent serves as the zero standard (blank) (0 nmol/ml).

Step 1: Leave all reagents out for 20 minutes. Prepare all reagents, working standards, blank and samples as directed in the previous sections. Pipette up and down each solution making sure they are homogenous.

Step 3：Pipette 50μl of standard to the standard wells leaving one well blank. Pipette 40μl of sample diluent to the remaining sample wells then add 10μl of sample (sample final dilution is 5-fold). Pipette sample to wells without touching the well wall and mix gently. Tap the bottom of the wells as needed to rid of the any bubbles.

Step 4: Incubate: Cover with the adhesive strip provided, incubate for 30 min at 37℃.

Step 5: Configurate liquid: Dilute wash solution 30-fold with distilled water.

Step 6: Washing: Uncover the adhesive strip, discard liquid, pipette washing buffer to every well, still for 2 minutes then drain, repeat 5 times. Flip the plate and aggressively tap all fluid out.

Step 7: Add enzyme: Pipette HRP-Conjugate reagent 50μl to each well, except blank well.

Step 8: Incubate: Operation with 4.

Step 9: Washing: Operation with 6.

Step10: Color: Pipette Chromogen Solution A 50ul and Chromogen Solution B 50ul to each well, avoid the light preservation for 15 min at 37℃.

Step 11: Stop the reaction: Pipette Stop Solution 50μl to each well, stop the reaction (the blue change to yellow).

Step 12: Read absorbance at 450nm after pipette Stop Solution within 15min.