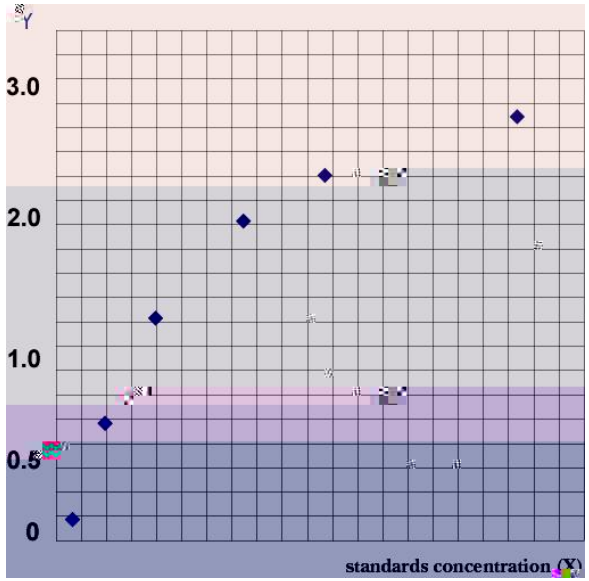


Y
3.0
2.0
1.0
0.5
0
standards concentration (X)

This TNF-Ŭ ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of TNF-Ŭ in the sample, the TNF-Ŭ ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus TNF-Ŭ concentration. T



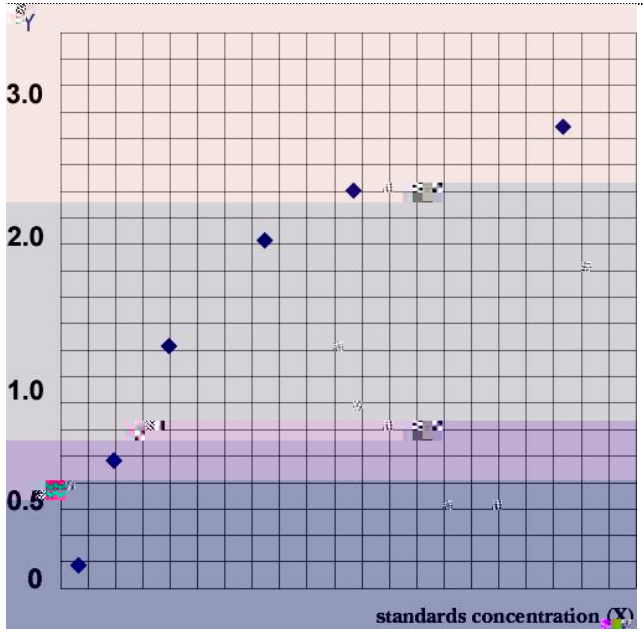
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Standard (S0 Ÿ S5) concentration was followed by:0,40,80,160,320,640 pg/ml.

20Ǐwash solution:Dilute with Distilled or deionized water 1:20.

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Add standard: Set Standard wells, testing sample wells. Add standard 50εl to standard well.
3. Add Sample: Add testing sample 10εl then add 40εl of Sample Diluent to testing sample well; Blank well doesnôt add anyting.
4. Add 100εl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37ÁC.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400εl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add chromogen solution A 50εl and chromogen solution B 50εl to each well. Gently mix and incubate for 15 minutes at 37ÁC.
7. Add 50εl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard before result interpretation. Construct the standard curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
5. The sensitivity by this assay is 1.0 pg/ml
6. Standard curve



Storage 2-8 .
validity six months.