ZyQuik™ sCD30 ELISA Kit

Cat. No. 99-0037
Size: 96 Tests
For Research Use Only
Lot No.

INTENDED USE

ZyQuik™ sCD30 ELISA is an enzyme-linked immunosorbent sandwich assay for quantitative detection of human sCD30 in cell culture lysates and supernatants, human whole blood or serum.

INTRODUCTION

The CD30 gene, localized at chromosome 1q36 [6], is closely linked to other members of the TNF receptor superfamily comprising TNF-receptors, nerve growth factor, CD40, APO-1/Fas, CD27, OX40 and the neurotrophin receptor. Interaction of the cytokine receptor CD30 with its ligand induces pleiotropic biologic effects, such as differentiation, activation, proliferation and cell death[8]. In $CD30^+$ ALCL cell lines, binding of CD30L induces apoptotic cell death.[8] CD30 seems to be involved in the control of the CD40/CD40L signal, T-cell proliferation and B-cell maturation induced by T-cell cytokines.[4] Thus, CD30 seems to transmit information that is essential for the immune response. In pathological conditions, CD30 positivity is regarded as a peculiar attribute of Hodgkin's and Reed-Sternberg cells.[2]

Increased levels of sCD3 are detected in the serum of patients with Hodgkin's disease[9], anaplastic large cell lymphomas (ALCL)[10] and embryonic carcinoma of the testis[11].Serum levels of sCD30 are also increased in most patients with HBsAg-positive chronic hepatitis and signs of active HBV replication, thus there is association of the raised sCD30 levels with the active phase of the illness.[5] Abnormal soluble CD30 serum accumulation has been reported in Omenn's syndrome, a severe immunodeficiency.[3] High elevations of sCD30 levels are found in patients of systemic lupus erythematosus, which correlates with disease activity [1], and in patients with rheumatoid arthritis[7].

REAGENTS AND MATERIALS PROVIDED

1 Assay Plate [coated with sCD30 monoclonal antibody (murine), sCD30 Standard curve (colored), lyophilized HRP-conjugated anti-sCD30 monoclonal antibody, and sample diluent]
2 sCD30 Standard curve (colored)
1 bottle (25 ml) 20X Wash Buffer (PBS with 1 % Tween 20)*
1 vial (15 ml) Substrate Solution (TMB)
1 vial (12 ml) Sample Diluent
1 vial (12 ml) Stop Solution (1M Phosphoric acid)
2 Adhesive Plate Cover
* Reagents containing thimerosal as preservative may be toxic if ingested

MATERIALS REQUIRED BUT NOT PROVIDED

5 ml and 10 ml graduated pipettes, beakers, flasks, and cylinders
10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
50 µl to 300 µl adjustable multichannel micropipette, disposable tips, and reservoir
Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

STORAGE

Store at -20°C

PRINCIPLE OF TEST

Protein of interest in the sample and standards binds to the antibody coated on the plate. A biotin-conjugated antibody is added and binds to protein captured by the first antibody. Streptavidin-HRP is added and binds to the biotin-conjugated antibody. The substrate solution is added to the wells to form the colored products. The reaction is then terminated by addition of acid and absorbance is measured at 450 nm. The protein concentration is determined from a standard curve.

PREPARATION OF REAGENTS

A. Wash Buffer: If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved. Add 50 ml of the 20x Wash Buffer into 950 ml distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should be adjusted to 7.4. Store at 2° to 25°C. The Wash Buffer is stable for 30 days.

TEST PROTOCOL

a. Determine the number of microwell strips required to test the desired number of samples plus microwell strip containing the blanks and standards (colored). Each sample and optional control sample should be assayed in duplicate. Extra Microwell Strips should be stored in foil bag with the desiccant provided at -20°C, and seal tightly.

b. Add 150 µl of distilled water in duplicate to all standard wells. (A1, A2 to G1, G2).

Figure 1. An example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (100 U/ml)	Standard 1 (100 U/ml)	Sample 1	Sample 1
B	Standard 2 (50 U/ml)	Standard 2 (50 U/ml)	Sample 2	Sample 2
C	Standard 3 (25 U/ml)	Standard 3 (25 U/ml)	Sample 3	Sample 3
D	Standard 4 (12.5 U/ml)	Standard 4 (12.5 U/ml)	Sample 4	Sample 4
E	Standard 5 (6.3 U/ml)	Standard 5 (6.3 U/ml)	Sample 5	Sample 5
F	Standard 6 (3.2 U/ml)	Standard 6 (3.2 U/ml)	Sample 6	Sample 6
G	Standard 7 (1.6 U/ml)	Standard 7 (1.6 U/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

c. Add 150 µl of distilled water in duplicate to the blank wells.

d. Add 140 µl of distilled water in duplicate, to the sample wells.

e. Add 10 µl of each Sample, in duplicate, to the designated wells.

f. Cover with a Plate Cover and incubate at room temperature for 3 hours, if available on a rotator set at 100 rpm.

g. Remove Plate Cover and empty wells. Wash the microwell strips three times with approximately 400 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. Take caution not to scratch the surface of the microwells. After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Do not allow the wells to dry.

h. Pipette 100 µl of TMB Substrate Solution to all wells, including the blank wells.

i. Incubate the microwell strips at room temperature (18° to 25°C) for about 15 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light. The point at which the substrate reaction is stopped is often determined by the ELISA reader being used. Many ELISA readers record absorbance only up to 2.0 O.D. Therefore the color development within individual microwells must be watched by the person running the assay, and the substrate reaction stopped before positive wells are no longer properly recordable.

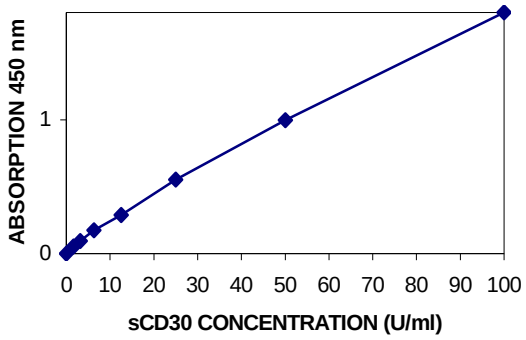
j. Stop the enzyme reaction by pipetting 100 µl of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

k. Read the absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the standards.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples. Duplicates should be within 20 per cent of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the Y-axis against the protein concentration on the X-axis. Draw a best-fit curve through the points of the graph.
3. The protein concentration for each sample can be determined using the standard curve.
4. For the samples, which have been diluted according to the instructions given in this manual, the concentration read from the standard curve must be multiplied by the dilution factor.
5. It is suggested that a control sample of known concentration is run in each assay.
6. A representative standard curve is shown in Figure 2. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 2. Standard curve of sCD30L



PERFORMANCE CHARACTERISTICS

A. Sensitivity

The limit of detection for sCD30 defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus three standard deviations) was determined to be 0.5 U/ml (mean of 6 independent assays).

B. Reproducibility

I. Intra-assay

Assay was carried out with 6 replicates of 6 samples containing different concentrations of sCD30. The overall intra-assay coefficient of variation was calculated to be 9.2 %.

Table 1. Intra-assay

Sample	Average Concentration (U/ml)	CV (%)
1	95	5.9
2	94.4	11.5
3	126.2	8.9
4	152.5	10.1
5	135.5	9.8

II. Inter-assay

Each assay was carried out with 6 replicates of 5 samples containing different concentrations of sCD30. The overall inter-assay coefficient of variation was calculated to be 12.9 %.

Table 2. Inter-assay

Sample	Average Concentration (U/ml)	CV (%)
1	94.9	10.7
2	94.4	17.5
3	126.2	9.4
4	151.6	16.2
5	135.5	10.7

C. Spike Recovery

The spike recovery was evaluated by spiking four levels of sCD30 into pooled normal human serum. Recoveries were determined in three independent experiments with 4 replicates each. The amount of endogenous sCD30 in unspiked serum was substracted from the spike values. Recoveries ranged from 74 to 111 % with an overall mean recovery of 93 %.

D. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a sCD30 positive serum. There was no detectable cross reactivity.

REFERENCES:

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RELATED PRODUCTS

Product Name	Clone	Cat. No.
Mouse anti-CD30	Ber-H2	08-0155
Mouse anti-CD30	Ber-H2	08-1155
Mouse anti-CD30	Ber-H2	18-7155

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