

## sCD30 ELISA (96x)

Enzyme-linked immunosorbent assay for quantitative detection of soluble human CD30

### Tradeform

[REF] 823 440 96 tests [IVD] complete test package

### Intend of use

The sCD30 ELISA is an enzyme-linked immunosorbent assay for in vitro Diagnosis [IVD] for the quantitative detection of human soluble CD30 (Ki-1) in cell culture supernatants, human serum, plasma, urine or other body fluids.

### Summary

Characterization of the CD30 (Ki-1) antigen has shown it to be in its mature form a transmembrane protein of about 120kDa (16) elaborated from an 84kD cytoplasmic precursor primarily through glycosylation (16). The extracellular domain of CD30, comprising 365 residues, has proved to be homologous to that of the TNF-receptor superfamily (1). The CD30 ligand (CD30L) has been identified, showing significant homology to TNF $\alpha$ , TNF $\beta$ , FasL, CD40L, CD27L and 4-1BBL (2). CD30L is expressed on activated T-cells (18). Interactions of the cytokine receptor CD30 with its ligand induces pleiotropic biologic effects, such as differentiation, activation, proliferation and cell death (10). 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 (4). There is growing evidence for a potential role of the CD30 molecule in clinical use and therapy (6). An 85kDa soluble form of the CD30 molecule (sCD30) has been shown to be released by CD30<sup>+</sup> cell in vitro and in vivo (11). Serum sCD30 detection can be regarded as a marker of the amount of CD30<sup>+</sup> cells present in the body. Increased serum levels of sCD30 have been reported for patients with CD30<sup>+</sup> ALCL (14) and CD30<sup>+</sup> embryonal carcinoma (13) of the testis and were found to correlate with the clinical phase of the disease, i.e. presentation complete remission (CR), relapse. Elevated serum values of sCD30 are shown in the majority of patients with Hodgkin's Disease (15) which again correlate with the presence of B symptoms and with the stage of the disease, i.e. tumor burden. While elevations of the soluble CD30 in serum of patients affected by infectious diseases usually are not detected, infectious mononucleosis is a notable exception (19). 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 (7). Abnormal soluble CD30 serum accumulation has been reported in Omenn's syndrome, a severe immunodeficiency (5). High elevations of sCD30 levels are found in patients of systemic lupus erythematosus which correlate with disease activity (3), in patients with the autoimmune liver disease primary biliary cirrhosis (12) and in patients with rheumatoid arthritis (9).

### Principle of test

An anti-sCD30 monoclonal coating antibody is adsorbed onto microtiter plate [MTP]. sCD30 present in the sample or standard binds to antibodies adsorbed to the [MTP]; a HRP-conjugated monoclonal anti-sCD30 antibody binds to sCD30 captured by the first antibody. Following incubation unbound enzyme conjugated anti-sCD30 is removed during a wash step of the [MTP] and substrate solution reactive with HRP is added to the wells. The kit is manufactured under the patent PCT/AT 02/00112 by Bender MedSystems GmbH.

### Kit contains

[MTP]	1	<b>Microwell Plate</b> Microwell plate coated with monoclonal antibody (murine) to human sCD30, HRP-Conjugate (anti-sCD30 mono-clonal (murine) antibody) and sample diluent, lyophilized.
[WB]	25 ml	<b>Wash buffer concentrate</b> 20 x (PBS with 1% Tween 20).
[SUB]	15 ml	<b>Substrate solution</b> tetramethyl-benzidine
[SD]	12 ml	<b>Sample diluent</b>
[STOP]	15 ml	<b>Stop solution</b> 1M phosphoric acid
[STD]	2	<b>Plate covers</b> adhesive
[FOL]	2	<b>aluminium pouches</b> with a sCD30 standard curve (coloured).
[I]	1	<b>package insert</b>

### Materials required but not provided

- 5 ml and 10 ml pipettes
- 10  $\mu$ l to 1,000  $\mu$ l adjustable single channel micropipettes with disposable tips
- 50  $\mu$ l to 300  $\mu$ l adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis.

### Warning and precautions

Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label. Do not expose kit reagents to strong light during storage or incubation. Do not pipette by mouth. Do not eat or smoke in areas where kit reagents or samples are handled. Avoid contact of skin or mucous membranes with kit reagents or specimens. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens. Avoid contact of substrate solutions with oxidizing agents and metal. Avoid splashing or generation of aerosols. In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes. Use clean, dedicated reagent trays for

dispensing substrate reagent. Glass-distilled water or deionized water must be used for reagent preparation. Substrate solutions must be at room temperature prior to use. Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite. The test must be performed by well-trained and authorised laboratory technicians. Testing is performed under aseptic and microbiologically controlled conditions. If the original package is damaged please inform the manufacturer.

### Storage instructions

Store ELISA plate or whole kit at -20°C. The [MTP] can also be removed, stored at -20°C, remaining kit reagents stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

### Specimen collection

Cell culture supernatants, human serum, plasma, urine or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive sCD30. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles. For stability and suitability of samples refer to 13. E, and F (see below).

### Preparation of reagents

#### Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved. Pour entire contents (25 ml) of the **Wash Buffer Concentrate** into a clean 500 ml graduated cylinder. Bring final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4. Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days.

#### Test protocol

Use plate immediately after removal from -20°C! Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water! Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results. Perform the washing step with at least 400 $\mu$ l of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results. Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells.

- Determine the number of microwell strips required to test the desired number of samples plus 1 microwell strip for blanks and standards (coloured). Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2.
- Add 150  $\mu$ l of distilled water in duplicate to all standard wells. (A1, A2 to G1, G2)
- Add 150  $\mu$ l of distilled water in duplicate to the blank wells.
- Add 125  $\mu$ l distilled water to all wells designated for samples.
- Add 25  $\mu$ l of each Sample, in duplicate, to the designated wells and mix the contents.
- Cover with a plate cover and incubate at room temperature (18° to 25°C) for 3 hours, if available on a rotator set at 100 rpm.
- Remove plate cover and empty wells. Wash the microwell strips three times with approximately 400  $\mu$ l Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.  
After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
- Pipette 100  $\mu$ l of TMB Substrate Solution to all wells, including the blank wells.
- Incubate the microwell strips at room temperature (18° to 25°C) for about 10 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 colour 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.
- Stop the enzyme reaction by quickly pipetting 100  $\mu$ l 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.
- Read absorbance of each microwell on a spectro-photometer 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 sCD30 standards.

### Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sCD30 concentration on the abscissa. Draw a best fit curve through the points of the graph.
- To determine the concentration of sCD30 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding sCD30 concentration.
- **\*Samples have been 1:4, thus the concentration read from the standard curve must be multiplied by the dilution factor (x4).**

- It is suggested that each testing facility establishes a control sample of known sCD30 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.
  - Every laboratory must prepare a standard curve for each group of microwell strips assayed.
- \* N.B: There is a common dilution factor for standards and samples due to the conjugate. However only the dilution factor given in this manual has to be considered for the calculation of sample concentrations.

#### Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of immunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or mouse monoclonal antibodies of irrelevant specificity) are added to the Sample.

#### 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

###### a. Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 5 serum samples containing different concentrations of sCD30. Two standard curves were run on each plate. The overall Intra-assay coefficient of variation has been calculated to be 9.2 %.

###### b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 5 serum samples containing different concentrations of sCD30. Two standard curves were run on each plate. The overall inter-assay coefficient of variation has been calculated to be 12.9%.

##### 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 subtracted from the spike values. Recoveries ranged from 74 to 111 % with an overall mean recovery of 93 %.

##### D. Dilution Parallelism

Four serum samples with different levels of sCD30 were assayed at four serial two-fold dilutions (1:4-1:32) with 4 replicates each. Recoveries ranged from 85% to 120% with an overall mean recovery of 97%.

##### E. Sample Stability

###### a. Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked with sCD30) were stored at -20°C and thawed and frozen several times, and the sCD30 levels determined. There was no loss of sCD30 by freezing and thawing for 5 times.

###### b. Storage Stability

Aliquots of a serum sample (spiked or unspiked with sCD30) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the sCD30 level determined after 24 h. There was no significant loss of sCD30 immunoreactivity during storage at above conditions.

##### F. Comparison of Serum and Plasma

From two individuals each, serum as well as EDTA, citrate, and heparin plasma was obtained at the same time and tested for sCD30. Concentrations were not significantly different and therefore all these blood preparations are suitable for use in the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

##### G. 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.

##### H. Expected Values

A panel of 32 sera from apparently healthy blood donors (male and female) was tested for sCD30. The detected sCD30 levels ranged between 17.5 and 130.7 U/ml with a mean level of 38.7 U/ml and a standard deviation of  $\pm 28.0$  U/ml.

#### Literature

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