KAMIYA BIOMEDICAL COMPANY

# Human sCD30 ELISA

**For the quantitative detection of human sCD30 in cell culture supernatant, serum and plasma**

## Cat. No. KT-030

**For research use only, not for use in diagnostic procedures.**

## PRODUCT INFORMATION

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## PRODUCT

The Human sCD30 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human sCD30. **The Human sCD30 ELISA is for research use only. Not for diagnostic or therapeutic procedures.**

## DESCRIPTION

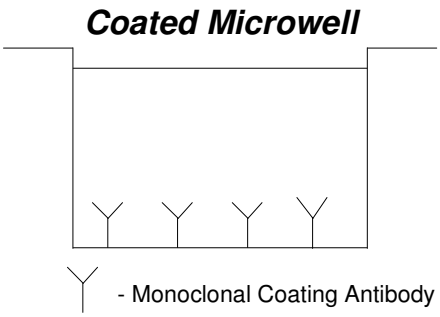
The CD30 (Ki-1) molecule was identified by a monoclonal antibody that was originally found to react with an epitope present in Hodgkin's and Reed-Sternberg cells in Hodgkin's disease. Later, the Ki-1 antigen was found to be consistently expressed by a subgroup of diffuse large-cell lymphomas that were called Ki-1 positive ($Ki\text{-}1^{+)}$ anaplastic large-cell lymphomas (ALCL).

Characterization of the CD30 antigen has shown it to be in its mature form a transmembrane protein of about 120 kDa elaborated from an 84 kDa cytoplasmic precursor primarily through glycosylation. The cloning of the CD30 gene has allowed the identification of a cDNA with an open reading frame predicting a 595 amino acid polypeptide. The extracellular domain of CD30, comprising 365 residues, has proved to be homologous to that of the TNF-receptor superfamily. The CD30 gene is localized at chromosome 1q36, 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. The CD30 ligand (CD30L) has been identified, showing significant homology to TNF$\alpha$, TNF$\beta$, FasL, CD40L, CD27L and 4-1BBl. CD30L is expressed on activated T-cells. Interactions of the cytokine receptor CD30 with its ligand induces pleiotropic biologic effects, such as differentiation, activation, proliferation and cell death. In $CD30^+$ ALCL cell lines binding of CD30L induces apoptotic cell death. CD30 furthermore seems to be involved in the control of the CD40/CD40L signal, T-cell proliferation and B-cell maturation induced by T-cell cytokines. Thus, CD30 seems to transmit information that is essential for the immune response. CD30 expression is strictly dependent on activation and proliferation of T- and B-cells. In pathological conditions, CD30 positivity is regarded as a peculiar attribute of Hodgkin's and Reed-Sternberg cells.

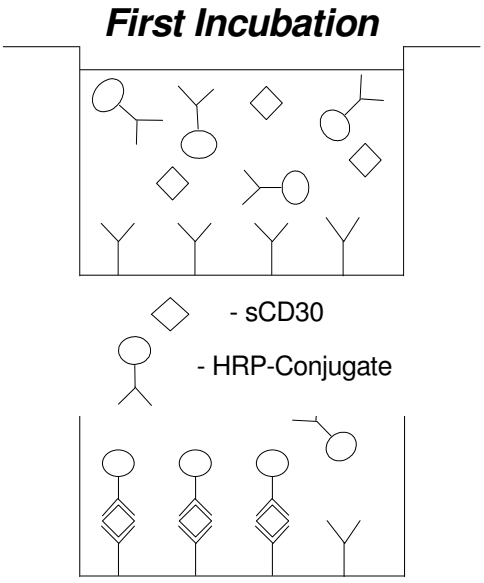
There is growing evidence for a potential role of the CD30 molecule in clinical use and therapy. An 85 kDa soluble form of the CD30 molecule (sCD30) has been shown to be released by $CD30^+$ cells in vitro and in vivo. It is probably derived from the 120 kDa membrane bound molecule by proteolytic cleavage. Serum sCD30 detection can be regarded as a marker of the amount of $CD30^+$ cells present in the body.

## PRINCIPLES OF THE TEST

An anti-human sCD30 coating antibody is adsorbed onto microwells.

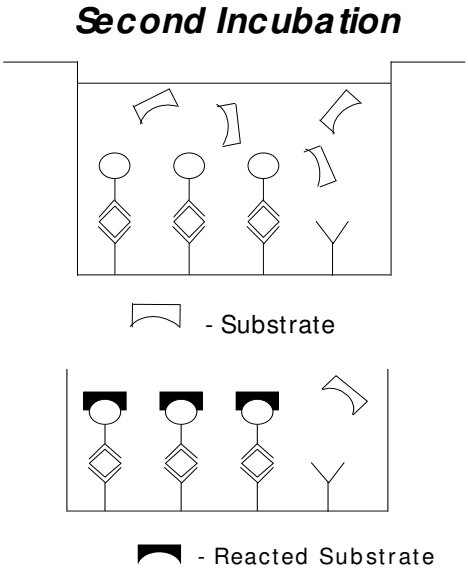



Human sCD30 present in the sample or calibrator binds to antibodies adsorbed to the microwells. A HRP-conjugated anti-human sCD30 antibody is added and binds to human sCD30 captured by the first antibody.




Following incubation, unbound HRP-conjugated anti-human sCD30 is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A colored product is formed in proportion to the amount of human sCD30 present in the sample or calibrator. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A calibration curve is prepared from 7 human sCD30 calibrator dilutions and human sCD30 concentration determined.



## COMPONENTS

- 1 aluminum pouch with a **Microwell Plate coated** with monoclonal antibody to human sCD30
- 1 vial (100 µL) **HRP-Conjugate** anti-human sCD30 monoclonal antibody
- 2 vials human sCD30 **Calibrator** lyophilized, 200 ng/mL upon reconstitution
- 1 vial **Control high**, lyophilized
- 1 vial **Control low**, lyophilized
- 1 bottle (50 mL) **Wash Buffer Concentrate**, 20x (PBS with 1% Tween 20)
- 1 vial (12 mL) **Sample Diluent**
- 1 vial (5 mL) **Assay Buffer Concentrate**, 20x (PBS with 1% Tween 20 and protein stabilizer)
- 1 vial (15 mL) **Substrate Solution** (tetramethyl-benzidine)
- 1 vial (15 mL) **Stop Solution** (1M Phosphoric acid)
- 1 vial (0.4 mL) **Blue-Dye**
- 1 vial (0.4 mL) **Green-Dye**
- 2 **Adhesive Films**

## STORAGE

Store kit reagents at 4°C except controls. Store lyophilized controls at -20°C. Immediately after use remaining reagents should be returned to cold storage (4°C), controls to -20°C, respectively. Expiration of the kit and reagents is stated on labels.

Expiration 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.

## Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL 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 de-ionized water
- Statistical calculator with program to perform regression analysis

## SPECIFICITY

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a human sCD30 positive serum. There was no crossreactivity detected.

## SPECIMEN COLLECTION

Cell culture supernatant, serum and plasma (EDTA, citrate, heparin) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or red cells as soon as possible after clotting and separation.

Pay attention to a possible **"Hook Effect"** due to high sample concentrations.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human sCD30. If samples are to be run within 24 hours, they may be stored at 4°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

## PROTOCOLS

**Preparation of Reagents**
**Buffer concentrates** should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the **Buffer Concentrates**, warm them gently until they have completely dissolved.

**A. Wash Buffer (1x)**

Pour entire contents (50 mL) of the **Wash Buffer Concentrate** (20x) into a clean 1,000 mL graduated cylinder. Bring to final volume of 1,000 mL with glass-distilled or de-ionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 4°C. Please note that the Wash Buffer (1x) is stable for 30 days. Wash Buffer (1x) may be prepared as needed according to the following table:

| Number of Strips | Wash Buffer Concentrate (20x) (mL) | Distilled Water (mL) |
|---|---|---|
| 1 - 6 | 25 | 475 |
| 1 - 12 | 50 | 950 |

**B. Assay Buffer (1x)**

Pour the entire contents (5 mL) of the **Assay Buffer Concentrate** (20x) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.
Store at 4°C. Please note that the Assay Buffer (1x) is stable for 30 days. Assay Buffer (1x) may be prepared as needed according to the following table:

| Number of Strips | Assay Buffer Concentrate (20x) (mL) | Distilled Water (mL) |
|---|---|---|
| 1 - 6 | 2.5 | 47.5 |
| 1 - 12 | 5.0 | 95.0 |

**C. HRP-Conjugate**

**Please note that the HRP-Conjugate should be used within 30 minutes after dilution.**
Make a 1:100 dilution of the concentrated **HRP-Conjugate** solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

| Number of Strips | HRP-Conjugate (mL) | Assay Buffer (1x) (mL) |
|---|---|---|
| 1 - 6 | 0.03 | 2.97 |
| 1 - 12 | 0.06 | 5.94 |

**D. Human sCD30 Calibrator**

Reconstitute **human sCD30 calibrator** by addition of distilled water. Reconstitution volume is stated on the label of the calibrator vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted calibrator = 200 ng/mL). Allow the calibrator to reconstitute for 10-30 minutes. Mix well prior to making dilutions. After usage remaining calibrator cannot be stored and has to be discarded. **Calibrator dilutions** can be prepared directly on the microwell plate or alternatively in tubes.

**E. Addition of Color-giving Reagents: Blue-Dye, Green-Dye**

In order to help our customers to avoid any mistakes in pipetting, KAMIYA BIOMEDICAL COMPANY offers a tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

**This procedure is optional**, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.

Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye***) can be added to the reagents according to the following guidelines:

**1. Diluent:** Before calibrator and sample dilution, add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

| | |
|---|---|
| 5 mL Sample Diluent | 20 µL ***Blue-Dye*** |
| 12 mL Sample Diluent | 48 µL ***Blue-Dye*** |
| 50 mL Sample Diluent | 200 µL ***Blue-Dye*** |

**2. HRP-Conjugate:** Before dilution of the concentrated HRP-Conjugate add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet: Preparation of HRP-Conjugate.

| | |
|---|---|
| 3 mL Assay Buffer (1x) | 30 µL ***Green-Dye*** |
| 6 mL Assay Buffer (1x) | 60 µL ***Green-Dye*** |
| 12 mL Assay Buffer (1x) | 120 µL ***Green-Dye*** |

**E. Control**

Reconstitute by adding 200 µL distilled water to lyophilized **controls** (10-30 minutes). Swirl or mix gently to insure complete and homogeneous solubilization. Further treat the controls like samples in the assay. For control range please refer to vial label. Store reconstituted controls aliquoted at -20°C. Avoid repeated freeze and thaw cycles.

## PROCEDURE

## TEST PROTOCOL

a. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and calibrators. Each sample, calibrator, 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 4°C sealed tightly.

b. Wash the microwell strips twice with approximately 400 µL **Wash** Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10-15 seconds** before aspiration. 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. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. **Do not allow wells to dry.**

c. **Calibrator dilution on the microwell plate** (Alternatively the calibrator dilution can be prepared in tubes): Add 100 µL of Sample Diluent in duplicate to all **calibrator wells**. Pipette 100 µL of prepared **calibrator** (see Preparation of Calibrator, concentration = 200.0 ng/mL) in duplicate into well A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of calibrator 1 = 100.0 ng/mL), and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human sCD30 calibrator dilutions ranging from 100.0 to 1.6 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

d. **External Calibrator Dilution**
Label 7 tubes, one for each calibrator point: C1, C2, C3, C4, C5, C6, C7. Then prepare 1:2 serial dilutions for the calibration curve as follows: Pipette 225 µL of Sample Diluent into each tube. Pipette 225 µL of reconstituted calibrator (concentration = 200 ng/mL) into the first tube, labeled C1, and mix (concentration of calibrator 1 = 100 ng/mL). Pipette 225 µL of this dilution into the second tube, labeled C2, and mix thoroughly before the next transfer. Repeat serial dilutions 5 more times thus creating the points of the calibration curve. Sample Diluent serves as blank.

In case of an **external calibrator dilution**, pipette 100 µL of these calibrator dilutions (C1-C7) in the calibrator wells according to Figure 1.

Figure 1. Table depicting an example of the arrangement of blanks, calibrators and samples in the microwell strips:

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **A** | Calibrator 1 (100.0 ng/mL) | Calibrator 1 (100.0 ng/mL) | Sample 1 | Sample 1 |
| **B** | Calibrator 2 (50.0 ng/mL) | Calibrator 2 (50.0 ng/mL) | Sample 2 | Sample 2 |
| **C** | Calibrator 3 (25.0 ng/mL) | Calibrator 3 (25.0 ng/mL) | Sample 3 | Sample 3 |
| **D** | Calibrator 4 (12.5 g/mL) | Calibrator 4 (12.5 ng/mL) | Sample 4 | Sample 4 |
| **E** | Calibrator 5 (6.3 ng/mL) | Calibrator 5 (6.3 ng/mL) | Sample 5 | Sample 5 |
| **F** | Calibrator 6 (3.1 ng/mL) | Calibrator 6 (3.1 ng/mL) | Sample 6 | Sample 6 |
| **G** | Calibrator 7 (1.6 ng/mL) | Calibrator 7 (1.6 ng/mL) | Sample 7 | Sample 7 |
| **H** | Blank | Blank | Sample 8 | Sample 8 |

e. Add 100 µL of **Sample Diluent** in duplicate to the **blank wells**.

f. Add 75 µL of **Sample Diluent** to the **sample wells**.

g. Add 25 µL of each **sample** in duplicate to the **sample wells**.

h. Prepare **HRP-Conjugate** (see Preparation of HRP-Conjugate).

i. Add 50 µL of **HRP-Conjugate** to all wells.

j. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 3 hours, if available on a microplate shaker set at 100 rpm.

k. Remove adhesive film and empty wells. **Wash** microwell strips 3 times according to point b. of the test protocol. Proceed immediately to the next step.

l. Pipette 100 µL of **TMB Substrate Solution** to all wells.

m. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 min. Avoid direct exposure to intense light. **The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.** It is recommended to add the stop solution when the highest calibrator has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Calibrator 1 has reached an OD of 0.9 – 0.95.

n. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well. 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 4°C in the dark.

o. 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 calibrators.

**Note: In case of incubation without shaking, the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.**

## CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate calibrators and samples. Duplicates should be within 20 per cent of the mean value.

- Create a calibration curve by plotting the mean absorbance for each calibrator concentration on the ordinate against the human sCD30 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human sCD30 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the calibration curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human sCD30 concentration.

- **If instructions in this protocol have been followed samples have been diluted 1:4 (25 µL sample + 75 µL Sample Diluent), the concentration read from the calibration curve must be multiplied by the dilution factor (x 4).**

- **Calculation of samples with a concentration exceeding calibrator 1 will result in incorrect, low human sCD30 levels (Hook Effect). Such samples require further external predilution according to expected human sCD30 values with Sample Diluent in order to precisely quantitate actual human sCD30 level.**

- It is suggested that each testing facility establishes a control sample of known human sCD30 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

Typical data using the human sCD30 ELISA

Measuring wavelength: 450 nm Reference wavelength: 620 nm

| Calibrator | Human sCD30 Concentration (ng/mL) | O.D. at 450 nm | Mean O.D. at 450 nm | C.V. (%) |
|---|---|---|---|---|
| 1 | 100.0 | 2.065<br>1.980 | 2.023 | 3.0 |
| 2 | 50.0 | 1.120<br>1.090 | 1.105 | 2.0 |
| 3 | 25.0 | 0.573<br>0.573 | 0.573 | 0.1 |
| 4 | 12.5 | 0.289<br>0.302 | 0.296 | 3.2 |
| 5 | 6.3 | 0.148<br>0.154 | 0.151 | 2.6 |
| 6 | 3.2 | 0.075<br>0.075 | 0.075 | 0.0 |
| 7 | 1.6 | 0.040<br>0.042 | 0.041 | 3.5 |
| Blank | 0 | 0.008<br>0.008 | 0.008 | 0 |

The OD values of the calibration curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

## LIMITATIONS

- Since exact conditions may vary from assay to assay, a calibration 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 at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, 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 radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

## PERFORMANCE CHARACTERISTICS

**A. Sensitivity**

The limit of detection of human sCD30 defined as the analyte concentration resulting in absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.3 ng/mL (mean of 6 independent assays).

**B. Reproducibility**

**a. Intra-assay**

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human sCD30. 2 calibration curves were run on each plate. Data below show the mean human sCD30 concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 4.1%.

| Sample | Experiment | sCD30 Conc. (ng/mL) | CV (%) |
|---|---|---|---|
| 1 | 1 | 82.9 | 2.6 |
| | 2 | 90.2 | 2.5 |
| | 3 | 71.2 | 5.5 |
| 2 | 1 | 43.7 | 3.9 |
| | 2 | 47.2 | 5.7 |
| | 3 | 46.0 | 4.5 |
| 3 | 1 | 28.6 | 1.4 |
| | 2 | 31.9 | 5.7 |
| | 3 | 29.8 | 2.6 |
| 4 | 1 | 25.1 | 5.5 |
| | 2 | 28.9 | 9.4 |
| | 3 | 29.5 | 3.3 |
| 5 | 1 | 98.4 | 2.0 |
| | 2 | 99.1 | 0.7 |
| | 3 | 91.3 | 2.7 |
| 6 | 1 | 50.7 | 3.4 |
| | 2 | 53.3 | 5.6 |
| | 3 | 52.3 | 6.3 |
| 7 | 1 | 34.1 | 2.8 |
| | 2 | 33.7 | 6.3 |
| | 3 | 32.5 | 1.7 |

**b. Inter-assay**

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human sCD30. 2 calibration curves were run on each plate. Data below show the mean human sCD30 concentration and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 5.6%.

| Sample | sCD30 Conc. (ng/mL) | CV (%) |
|---|---|---|
| 1 | 81.4 | 11.8 |
| 2 | 45.6 | 3.9 |
| 3 | 30.1 | 5.7 |
| 4 | 27.8 | 8.5 |
| 5 | 96.3 | 4.5 |
| 6 | 52.1 | 2.5 |
| 7 | 33.4 | 2.5 |

**C. Spike Recovery**

The spike recovery was evaluated by spiking 4 levels of human sCD30 into pooled human serum. Recoveries were determined in 2 independent experiments with 8 replicates each.

The amount of endogenous human sCD30 in unspiked serum was subtracted from the spike values. The recovery ranged from 95% to 115% with an overall mean recovery of 104%.

**D. Dilution Parallelism**

4 serum samples with different levels of human sCD30 were analyzed at serial 2 fold dilutions with 4 replicates each. The recovery ranged from 90% to 106% with an overall mean recovery of 99%.

| Sample | Dilution | sCD30 Concentration (ng/mL) Expected Value | Observed Value | % Recovery of Exp. Value |
|---|---|---|---|---|
| 1 | 1:4 | -- | 83.7 | -- |
| | 1:8 | 41.8 | 38.8 | 93 |
| | 1:16 | 20.9 | 20.7 | 99 |
| | 1:32 | 10.5 | 10.7 | 102 |
| 2 | 1:4 | -- | 50.0 | -- |
| | 1:8 | 25.0 | 22.7 | 91 |
| | 1:16 | 12.5 | 13.3 | 106 |
| | 1:32 | 6.3 | 6.4 | 102 |
| 3 | 1:4 | -- | 75.4 | -- |
| | 1:8 | 37.7 | 33.8 | 90 |
| | 1:16 | 18.8 | 18.9 | 100 |
| | 1:32 | 9.4 | 10.0 | 106 |
| 4 | 1:4 | -- | 85.3 | -- |
| | 1:8 | 42.7 | 39.7 | 93 |
| | 1:16 | 21.3 | 20.9 | 98 |
| | 1:32 | 10.7 | 11.7 | 106 |

**E. Sample Stability**

**a. Freeze-Thaw Stability**

Aliquots of serum samples (spiked or unspiked) were stored at -20℃ and thawed 5 times, and the human sCD30 levels determined. There was no significant loss of human sCD30 immunoreactivity detected by freezing and thawing.

**b. Storage Stability**

Aliquots of serum samples (spiked or unspiked) were stored at -20℃, 4℃, room temperature (RT) and at 37℃, and the human sCD30 level determined after 24 h. There was no significant loss of human sCD30 immunoreactivity detected during storage under above conditions.

## Warnings and precautions

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it be used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
- 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 solution 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 the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or de-ionized water must be used for reagent preparation.
- Substrate solution 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℃.
- 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.

**FOR RESEARCH USE ONLY**

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