ELISA Kit
Catalog #KHS6031

Human
sCD30

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INTRODUCTION

Characterization of the CD30 antigen has shown it to be in its mature form, a transmembrane protein of about 120 kDa (12,22) elaborated from an 84 kDa cytoplasmic precursor primarily through glycosylation (22). The cloning of the CD30 gene has allowed the identification of a cDNA with an open reading frame predicting a 595 amino acid polypeptide (7). The extracellular domain of CD30, comprising 365 residues, has proved to be homologous to that of the TNF-receptor superfamily (1). The CD30 gene is localized at chromosome 1q36 (11), 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-α, TNF-β, FasL, CD40L, CD27L and 4-1BBl (2,9,25). CD30L is expressed on activated T-cells (24). Interactions of the cytokine receptor CD30 with its ligand induces pleiotropic biologic effects, such as differentiation, activation, proliferation and cell death (14). In CD30+ ALCL cell lines, binding of CD30L induces apoptotic cell death (14). 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 (6). 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 (27). It is probably derived from the 120 kDa membrane bound molecule by proteolytic cleavage (15). Serum sCD30 detection can be regarded as a marker of the amount of CD30+ cells present in the body.

PURPOSE

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

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

An anti-sCD30 monoclonal coating antibody is adsorbed onto microwells. sCD30 present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-sCD30 antibody is added and binds to sCD30 captured by the first antibody. Following incubation, unbound enzyme conjugated anti-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 sCD30 present in the sample. The reaction is terminated by addition of stop solution and the absorbance is measured at 450 nm. A standard curve is prepared from seven sCD30 standard dilutions and sCD30 sample concentration determined.

REAGENTS PROVIDED

Note: Store all reagents at 2 to 8°C.

Reagent	***96 Test Kit***
Aluminum pouch with *Microwell Plate* coated with monoclonal antibody (murine) to human sCD30.	1 plate
HRP-Conjugate, anti-sCD30 monoclonal (murine) antibody. Contains 0.01% Proclin® 300; 0.1 mL per vial.	1 vial
Human sCD30 Standard Concentrate (200 U/mL upon reconstitution), lyophilized. Contains 0.01% Proclin® 300.	2 vials
Wash Buffer Concentrate (20x) (PBS with 1% Tween 20). Contains 0.01% Proclin® 300; 50 mL per bottle.	1 bottle
Sample Diluent (buffered protein matrix). Contains 0.01% Proclin® 300; 12 mL per bottle.	1 bottle
Assay Buffer Concentrate (20x) (PBS with 1% Tween 20 and 10% BSA). Contains 0.01% Proclin® 300; 5 mL per vial.	1 vial
Substrate Solution; 15 mL per vial.	1 vial
Stop Solution (1 M Phosphoric Acid); 15 mL per bottle.	1 bottle
Blue-Dye. Contains 0.01% Proclin® 300; 0.4 mL per vial.	1 vial
Green-Dye. Contains 0.01% Proclin® 300; 0.4 mL per vial.	1 vial
Plate Covers, adhesive strips.	2 strips

STORAGE INSTRUCTIONS

Store kit reagents at 2 to 8°C. Immediately after use, remaining reagents should be returned to cold storage (2 to 8°C). Expiration date of the kit and reagents is stated on box label.

The expiration date 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 and plasma, or other biological samples are suitable for use in the assay. Remove the serum or plasma 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.

Samples should be kept at 2 to 8°C and separated rapidly (within half an hour) 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.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. 5 mL and 10 mL graduated pipettes.
2. 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips.
3. 50 µL to 300 µL adjustable multi-channel micropipette with disposable tips.
4. Multi-channel micropipette reservoir.
5. Beakers, flasks, cylinders necessary for preparation of reagents.
6. Device for delivery of wash solution (multi-channel micropipette, wash bottle or automatic wash system).
7. Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wavelength).
8. Glass-distilled or deionized water.
9. Statistical calculator with program to perform linear regression analysis.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. Do not mix or substitute reagents with those from other lots or sources.
2. Do not use kit reagents beyond expiration date on label.
3. Do not expose kit reagents to strong light during storage or incubation.
4. Do not pipette by mouth.
5. Do not eat or smoke in areas where kit reagents or samples are handled.
6. Avoid contact of skin and mucous membranes with kit reagents or specimens.
7. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
8. Avoid splashing or generation of aerosols.

9. Avoid contact of substrate solution with oxidizing agents and metal.
10. 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.
11. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
12. Exposure to acids will inactivate the conjugate.
13. Glass-distilled water or deionized water must be used for reagent preparation.
14. Substrate solution must be at room temperature prior to use.
15. Decontaminate and dispose of 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.
16. 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.

SAFETY

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 is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory coats, 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. See material safety data sheet(s) and/or safety statement(s) for specific advice.

PREPARATION OF REAGENTS

Wash Buffer (reagent A.) and Assay Buffer (reagent B.) should be prepared before starting with the test procedure.

A. Wash Buffer

If crystals have formed in the *Wash Buffer Concentrate*, warm it gently until they have completely dissolved.

Pour entire contents (50 mL) of the *Wash Buffer Concentrate* into a clean 1,000 mL graduated cylinder. Bring final volume to 1,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. Adjust the pH of the final solution 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. *Wash Buffer* may be prepared as needed according to the following table:

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

B. Assay Buffer

Mix the contents of the bottle well. Add contents of *Assay Buffer Concentrate* (5.0 mL) to 95 mL distilled or deionized water and mix gently to avoid foaming. Store at 2 to 8°C. Please note that the *Assay Buffer* is stable for 30 days. *Assay Buffer* may be prepared as needed according to the following table:

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

C. Preparation of HRP-Conjugate

Dilute the *HRP-Conjugate* 1:100 just prior to use with *Assay Buffer* (reagent B) in a clean plastic tube. Mix the contents of the tube well.

Please note that the *HRP-Conjugate* should be used within 30 minutes after dilution. The dilution (1:100) of the *HRP-Conjugate* may be prepared as needed according to the following table:

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

D. Preparation of sCD30 Standard

Reconstitute *sCD30 Standard* by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Mix gently to insure complete reconstitution.

Prepare Standard shortly before use. Use immediately after reconstitution. **Do not store reconstituted Standard**.

E. Addition of color-producing reagents: Blue-Dye, Green-Dye

In order to help our customers to avoid any mistakes in pipetting the ELISAs, we offer a new 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. It does not in any way interfere with the test results. It 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) Sample Diluent: Before 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 Diluent	20 µL Blue-Dye
12 mL Diluent	48 µL Blue-Dye

(2) HRP-Conjugate: Before dilution of the concentrated conjugate, add the Green-Dye at a dilution of 1:100 (see table below) to the *Assay Buffer* 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	30 µL Green-Dye
6 mL Assay Buffer	60 µL Green-Dye
12 mL Assay Buffer	120 µL Green-Dye

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the Procedural Notes/Lab Quality Control section before carrying out the assay.

1. Mix all reagents thoroughly, without foaming, before use.
2. Determine the number of *Microwell Strips* required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra *Microwell Strips* coated with monoclonal antibody (murine) to human sCD30 from holder and store in foil bag with the desiccant provided at 2 to 8°C sealed tightly.
3. Wash the *Microwell Strips* twice with approximately 300 µL *Wash Buffer* per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
4. 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 no longer than 15 minutes. Do not allow wells to dry.

5. Add 100 µL of *Sample Diluent*, in duplicate, to all standard wells. Prepare standard dilutions by pipetting 100 µL of solubilized *sCD30 Standard*, in duplicate, into wells A1 and A2 (see Figures 1 and 2). Mix the contents of wells A1 and A2 by repeated aspiration and ejection and transfer 100 µL to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of *sCD30 Standard* dilutions ranging from 100 to 1.6 U/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of *sCD30 Standard* dilutions:

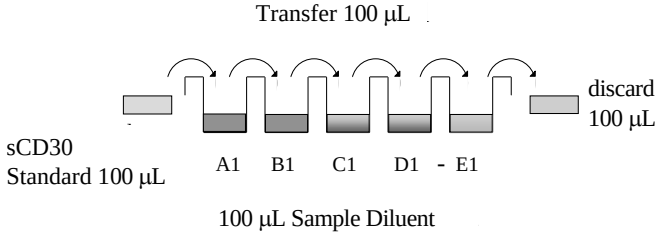


Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the *Microwell Strips*:

	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

6. Add 100 µL of *Sample Diluent*, in duplicate, to the blank wells.
7. Add 75 µL of *Sample Diluent* to all wells designated for samples.
8. Add 25 µL of each sample, in duplicate, to the designated wells and mix contents.
9. Prepare *HRP-Conjugate*. (Refer to PREPARATION OF REAGENTS)
10. Add 50 µL of diluted *HRP-Conjugate* to all wells, including the blank wells.
11. Cover with a *Plate Cover* and incubate at room temperature (18 to 25°C) for 3 hours on a rotator set at 100 rpm, if available.

12. Remove *Plate Cover* and empty wells. Wash *Microwell Strips* 3 times according to point 3. of the test protocol. Proceed immediately to the next step.
13. Pipette 100 µL of *TMB Substrate Solution* into all wells, including the blank wells.
14. Incubate the *Microwell Strips* at room temperature (18 to 25°C) for about 10 minutes on a rotator set at 100 rpm, if available. Avoid direct exposure to intense light. **The color development on the plate should be monitored and the substrate reaction stopped (see point o. of this protocol) before positive wells are no longer properly recorded.** It is recommended to add the stop solution when the highest standard 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 an O.D. of 0.6 - 0.65 is reached.
15. Stop the enzyme reaction by quickly 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 to 8°C in the dark.
16. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 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 the samples and the human sCD30 standards.

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

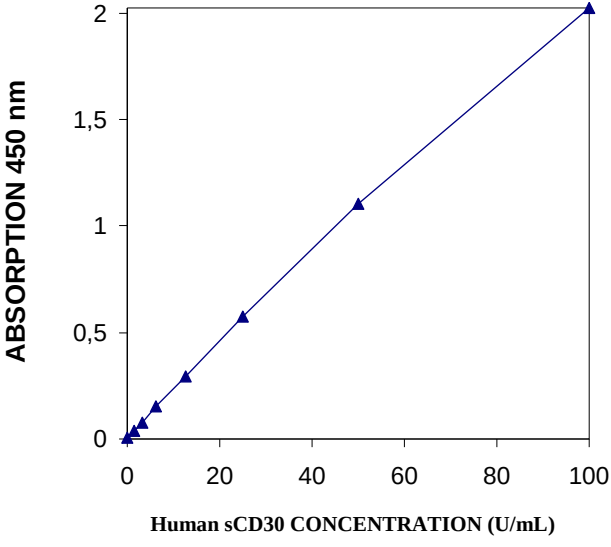
1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the sCD30 concentration on the abscissa. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating 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.
4. For samples which have been diluted according to the instructions given in this manual 1:4, the concentration read from the standard curve must be multiplied by the dilution factor (x4).

Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD30 levels. Such samples require further dilution of 1:8 - 1:16 with *Sample Diluent* in order to precisely quantitate the actual sCD30 level.

5. It is suggested that each testing facility establish a control sample of known sCD30 concentration and run 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.
6. A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of *Microwell Strips* assayed.

Figure 3. Representative standard curve for sCD30 ELISA. sCD30 was diluted in serial two-fold steps in *Sample Diluent*, symbols represent the mean of three parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of *Microwell Strips* assayed.



Typical data using the sCD30 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	sCD30 concentration (U/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	100	2.065	2.023	3.0
	100	1.980		
2	50	1.120	1.105	2.0
	50	1.090		
3	25	0.573	0.573	0.1
	25	0.573		
4	12.5	0.289	0.296	3.2
	12.5	0.302		
5	6.3	0.148	0.151	2.6
	6.3	0.154		
6	3.2	0.075	0.075	0.0
	3.2	0.075		
7	1.6	0.040	0.041	3.5
	1.6	0.042		
Blank	0	0.008	0.008	
	0	0.008		

LIMITATIONS OF THE PROCEDURE

1. Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
4. Improper or insufficient washing at any stage of the procedure 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.
5. The use of radioimmunotherapy 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 analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the *Sample Diluent*.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection for sCD30 defined as the analyte concentration resulting in an absorption significantly higher than the absorption of the dilution medium (mean plus two standard deviations) was determined to be 0.33 U/mL (mean of 6 independent assays).

REPRODUCIBILITY

1. Intra-Assay

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

Positive Sample	Experiment	sCD30 Concentration (U/mL)	Coefficient of Variation (%)
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

2. 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 7 serum samples containing different concentrations of sCD30. Two standard curves were run on each plate. Data below show the mean sCD30 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 5.6%.

Sample	sCD30 Concentration (U/mL)	Coefficient of Variation (%)
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

SPIKE RECOVERY

The spike recovery was evaluated by spiking four levels of sCD30 into pooled normal human serum. As shown below recoveries were determined in two independent experiments with 8 replicates each. The amount of endogenous sCD30 in unspiked serum was subtracted from the spike values. Recoveries ranged from 95 to 115% with an overall mean recovery of 104%.

sCD30 Spike (U/mL)	Experiment	Recovery % sCD30
80	1	102
	2	115
40	1	101
	2	108
20	1	95
	2	107
10	1	99
	2	103

DILUTION PARALLELISM

Four serum samples with different levels of sCD30 were assayed at four serial two-fold dilutions (1:4 to 1:32) with 4 replicates each. In the table below the percent recovery of expected values is listed. Recoveries ranged from 90 to 106% with an overall mean recovery of 99%.

		sCD30 Concentration (U/mL)		
Sample	Dilution	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

SAMPLE STABILITY

1. 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 significant loss of sCD30 by freezing and thawing for 5 times.

2. Storage Stability

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

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.

SPECIFICITY

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

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 ± 28.0 U/mL.

REAGENT PREPARATION SUMMARY

A. Wash Buffer Add Wash Buffer Concentrate (20x) 50 mL to 950 mL distilled water.

B. Assay Buffer

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

C. HRP-Conjugate

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

D. Standard Reconstitute sCD30 Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

ASSAY METHOD SUMMARY

1. Wash *Microwell Strips* twice with *Wash Buffer*.
2. Add 100 µL *Sample Diluent*, in duplicate, to all standard wells.
3. Pipette 100 µL solubilized *sCD30 Standard* into the first wells and create standard dilutions ranging from 100 to 1.6 U/mL by transferring 100 µL from well to well. Discard 100 µL from the last wells.
4. Add 100 µL *Sample Diluent*, in duplicate, to the blank wells.
5. Add 75 µL *Sample Diluent*, in duplicate, to sample wells.
6. Add 25 µL Sample, in duplicate, to designated wells.
7. Prepare *HRP-Conjugate*.
8. Add 50 µL of diluted *HRP-Conjugate* to all wells.
9. Cover *Microwell Strips* and incubate 3 hours at room temperature (18 to 25°C).
10. Empty and wash *Microwell Strips* 3 times with *Wash Buffer*.
11. Add 100 µL of *TMB Substrate Solution* to all wells, including blank wells.
12. Incubate the *Microwell Strips* for about 10 minutes at room temperature (18 to 25°C).
13. Add 100 µL *Stop Solution* to all wells including blank wells.
14. Blank microwell reader and measure color intensity at 450 nm.

Note: For samples which have been diluted according to the instructions given in this manual 1:4, the concentration read from the standard curve must be multiplied by the dilution factor (x4). Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low sCD30 levels. Such samples require further dilution of 1:8 to 1:16 with Sample Diluent in order to precisely quantitate the actual sCD30 level.

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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