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Human sCD27 ELISA Kit

Catalog No: M1960 **Size:** 3 x 96 tests

I. Introduction

CD27 is a lymphocyte specific member of a growing family of receptors for membrane bound and soluble cytokines, the TNFr super family. Next to the type I and type II TNFr (CD120a and CD120b) this receptor family comprises the Hodgkin's disease-related CD30 antigen, CD40, FAS/APO-1 (CD95), 4-1BB and the rat T-cell surface molecule OX40, all proteins that are predominantly expressed on cells of the haemapoetic system. The expression of CD27 is restricted to cells of the lymphoid lineage and appears to be related to functional differentiation programs of T- and B-cells. With respect to the T-cell compartment, thymocytes at the $CD3^{low}$ stage do not express CD27, but expression can be induced after activation. CD27 becomes constitutively expressed on $CD3^{high}$ medullary thymocytes and this expression is further enhanced upon activation. Thymic emigrants express CD45RA along with CD27 and these naive T-cells have a very strong capacity to upregulate CD27 after TCR/CD3 triggering. In addition, activation induces the production of a soluble 32 kDa form of CD27, which is most likely generated by the proteolytic cleavage of the putative ligand binding domain of the 55 kDa trans membrane molecule. This soluble receptor molecule (sCD27) can not only be found in supernatants of activated lymphocytes, but is also present in biological fluids of healthy individuals. Levels of sCD27 have been found to increase in patients suffering from a variety of immunopathological diseases which suggests that measurement of sCD27 can serve as a marker for T-cell activation *in vivo*.
This PeliKine compact™ human sCD27 ELISA kit has been developed for fast, reproducible and specific quantification of human sCD27 in serum and in plasma, as well as in cell-culture supernatant.

II. Principle of the Test

The PeliKine compact™ human sCD27 ELISA kit is a "sandwich-type" of enzyme immunoassay in which a monoclonal CD27 antibody is coated to polystyrene microtiter wells. Soluble CD27 molecules (sCD27), present in a measured volume of sample or standard are bound by the antibodies on the microtiter plate, and non-bound material is removed by washing. Subsequently, a biotinylated second monoclonal CD27 antibody is added. This antibody binds to the sCD27-antibody complex present in the microtiter well. Excess biotinylated antibody is removed by washing, followed by addition of horseradish peroxidase (HRP) conjugated streptavidin, which binds onto the biotinylated side of the sCD27-antibody sandwich. After removal of non-bound streptavidine-HRP conjugate by washing, substrate solution is added to the wells. A colored product is formed in proportion to the amount of sCD27 present in the sample or standard. After the reaction has been terminated by the addition of a stop solution, absorbance is measured in a microtiter plate reader. From the absorbance of samples and those of a standard curve, the concentration of sCD27 can be determined by interpolation with the standard curve.
The PeliKine compact™ sCD27 ELISA kit is intended for research purposes only.

III. STORAGE AND STABILITY

The Pelikine compact™ sCD27 ELISA kit should be stored at -18°C to -32°C. The performance of the kit is guaranteed until the expiration date shown on the case label.

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IV. CONTENTS OF THE KIT

The PeliKine compact™ human sCD27 ELISA kit contains material sufficient for 288 tests, including standard curve samples. The reagents provided are:

Quantity	Kit component		Volume	Cap
1 vial	coating antibody	100-fold concentrated	375 µl	red
1 vial	Standard	400 U/ml	1000 µl	black
1 vial	biotinylated antibody	100-fold concentrated	375 µl	yellow
1 vial	streptavidin-HRP conjugate	1,000-fold concentrated	50 µl	brown
1 bottle	dilution buffer	10- fold concentrated	50 ml	
3 pcs	microtiter plate + lid	-	-	
10 pcs	plate seals	-	-	

V. PRECAUSTIONS FOR USE

1) The PeliKine compact™ sCD27 ELISA kit is intended for research purposes only.
2) Only use the reagents and microtiter plates supplied with the kit, do not mix reagents from different kit lots.
3) Handle all plasma and serum samples with care to prevent transmission of blood-born infections.
4) Sodium azide inactivates HRP, do **not** use sodium azide-containing solutions, nor add sodium azide to the supplied materials
5) All reagents contain merthiolate (0.001 % w/v).
6) The sCD27 standard contains human serum which has been found to be non-reactive for Hepatitis B surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV). Nevertheless the standard should be handled as potentially hazardous and capable of transmitting diseases.
7) Centrifuge all vials before use (1 minute 3000 x g).
8) With the exception of the substrate blank wells, do not allow wells to stand uncovered or dry for extended periods between incubation steps.

VI. ADDITIONAL BUFFERS & SOLUTIONS REQUIRED

Additional materials required

- Pipetting devices for accurate delivery of 1-10 ml, 50 ml, 100 ml and 1 ml volumes.
- Beakers, flasks, cylinders necessary for preparation of reagents.
- Device for delivery of washing buffer (wash bottle / automated plate washer).
- Microtiter plate reader.

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Additional buffers and solutions

PBS stock solution [20 x]: 0.2 M Phosphate Buffered Saline (PBS)

Dissolve	32 g	Na2HPO4.2H2O
	6 g	NaH2PO4.2H2O
	164 g	NaCl

in 900 ml distilled water
(intensive stirring and some heating will speed dissolution).
Bring the temperature of the solution back to room temperature (18-25°C) and check pH; if necessary adjust pH to 6.8- 6.9 with concentrated HCl or NaOH, and add distilled water to a volume of 1 liter (when diluted 20 times the obtained buffer will have a pH of 7.2 - 7.4).
Add 20 mg merthiolate as preservative. Do **not** use sodium azide (NaN_3) since this preservative reduces the quality of the enzymatic label.

The prepared buffer can be stored up to three months at 2-8°C.

Note: in the concentrated buffer salt crystals may appear when stored at 2-8°C. Before preparing the working-strength buffer, first warm the concentrated buffer BRIEFLY to 37°C to dissolve the precipitate.

Washing buffer: PBS with 0.005% TWEEN20

Make 1 liter of working-strength PBS by diluting the PBS stock solution (see above) 20 fold with distilled water.

Add 50 µl TWEEN 20.

The prepared buffer can be stored up to one month at 2-8°C.

Substrate buffer: 0.11 M acetate buffer pH 5.5

Dissolve 15.0 g sodium-acetate ($CH_3COONa.3H_2O$) in 800 ml distilled water.
Adjust pH to 5.5 with glacial acetic acid, add distilled water to a volume of liter.
Do not add any preservative (e.g. merthiolate, sodium azide) since this may affect the quality of the enzymatic color development.
The prepared buffer can be stored up to two weeks at 2-8°C.

3,5,3',5'-tetramethylbenzidine (TMB) stock solution: 6 mg/ml TMB in DMSO

Dissolve 30 mg 3,5,3',5'-tetramethylbenzidine (TMB) in 5 ml dimethylsulfoxide (DMSO).
The prepared stock solution can be stored up to 1 month at room temperature (18-25°C) and protected against light.

Hydrogen peroxide stock solution: 3% H_2O_2 solution in distilled water.

The prepared stock solution can be stored up to one month at 2-8°C.

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Substrate solution:

For each plate mix the following reagents:

12 ml substrate buffer
200 µl TMB stock solution
12 µl H_2O_2 stock solution

The substrate solution should be prepared just before use and has to be at room temperature (18-25°C) for optimal reproducible results.

Stop solution: 1.8 M H_2SO_4 solution in distilled water.

VII. ADDITIONAL INFORMATION

Sensitivity
MEAN calculated zero signal + 3 SD : 1.0 U/ml
2 x (MEAN calculated zero signal) : 4.0 U/ml

Note: the sensitivity is dependent upon the type and quality of enzymatic substrate.

Expected values
sCD27 values in fresh serum and plasma samples of healthy individuals are below 350 U/ml (Mean + 3x SD)

VIII. ASSAY PROCEDURE

*1. **BRING ALL REAGENT TO ROOM REMPERATURE (18-25°C)**,* with the exception of the streptavidin-HRP conjugate which has to be kept at -18°C to -32°C to ensure stability. Centrifuge all vials before use (1 minute 3000 x g).

For your convenience an easy-reference manual with check list is available on the last page of this leaflet.

2. DILUTION BUFFER
The kit contains one bottle with 10-fold concentrated dilution buffer.
For optimal assay results, dilute samples and standards in working-strength dilution buffer.

Calculate the quantity of dilution buffer required (approximately 12 ml undiluted buffer per microtiter plate) and prepare a working-strength solution by diluting the opalescent concentrated buffer 10 times in distilled water before use. The working-strength dilution buffer can be stored for up to one week at 2-8°C.

3. MICROTITER PLATES **Coating antibody**
Coating
The kit contains three micotiter plates for 96 tests each, including the standard curve samples.

Prepare working-strength PBS (1:20 dilution of stock PBS as described on page 3 of the information leaflet.

Per microtiter plate, add 120 µl of coating antibody (red-capped vial) to 12 ml working-strength PBS.

Add 100 µl to all wells, cover microtiter plate(s) with lid and **incubate overnight at room temperature (18-25°C).**

Washing procedure

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Prepare working-strength PBS (1:20 dilution of stock PBS as described in the leaflet).

Aspirate supernatants from wells and completely fill the wells (> 300 µl) with working-strength PBS and aspirate. Repeat this four times, after the final aspiration the wells should be dry.

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Blocking procedure

Add 200 µl working-strength dilution buffer to all wells, cover microtiter plate(s) with adhesive seal, gently agitate the microtiter plate by tapping the edge of the plate for a few seconds to mix contents of each well and **incubate for 30 minutes at room temperature (18-25°C)**.

4. sCD27 STANDARD **Standard curve preparation**

The kit contains one black-capped vial with 400 U/ml sCD27.

Label 7 tubes, one tube for each dilutions: 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 U/ml. Pipette 450 µl of working strength dilution buffer into the tube labeled 100 U/ml and 300 µl of working strength dilution buffer into the other tubes.
Transfer 150 µl of the sCD27 standard into the first tube labeled 100 U/ml, mix well and transfer 300 µl of this dilution into the second tube labeled 50 U/ml.
Repeat the serial dilutions five more times by adding 300 µl of the previous tube of diluted standard to the 300 µl of dilution buffer.
The standard curve will contain 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0 pg/ml (dilution buffer).

It is recommended to prepare two separate series for each assay.

Avoid repeated freeze-thawing of the standard, although experimental data have shown that up to 3 freeze-thaw cycles have no effect on the sCD27 levels of the standard.

5. SAMPLES

Serum, EDTA-anti-coagulated plasmas, and culture fluids are suitable for use in the assay. (caution: separate plasma/serum and blood cells within 4 hours after collection). Do not use grossly hemolyzed or lipemic specimens. If samples are to be run within 24 hours, they may be stored at 2-8°C; otherwise samples should be stored frozen (below -18°C).

Up to 3 freeze-thaw cycles have no effect on the sCD27 levels of serum or plasma samples. Nonetheless, excessive freeze-thaw cycles should be avoided. Prior to the assay, frozen samples should be thawed **as quickly as possible** in a 37°C water bath and then brought to room temperature (18-25°C).

It is recommended to dilute the test samples at least 1:10 in working-strength dilution buffer. If high levels of sCD27 (>800 pg/ml) are expected in the test samples, additional dilutions of sample i.e. 1:50 and 1:100 should also be prepared.

6. FIRST WASH STEP

Prepare washing buffer as described on page 3 of this leaflet.

Wash the required microtiter plates five times with washing buffer in a plate washer.
In case of manual washing, completely fill the wells (> 300 µl) with washing buffer and aspirate, repeat this four times. After the final aspiration the wells should be dry.

7. FIRST INCUBATION STEP **Standards and samples**

Leaving the substrate blank wells empty, transfer 100 µl of the prepared standards and samples in duplicate into the appropriate wells (see recommended plate plan).
Cover plate(s) with adhesive seal, gently agitate the microtiter plate by tapping the edge of the plate for a few seconds to mix contents of each well and incubate for 1 hour at room temperature (18-25°C).

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8. SECOND WASH STEP

Aspirate supernatant from wells and wash the microtiter plate(s) as described in point 6 above.

9. SECOND INCUBATION STEP **biotinylated antibody**

The kit contains one yellow-capped vial with biotinylated antibody

Per microtiter plate, add 120 µl biotinylated antibody to 12 ml working-strength dilution buffer just before use.

Leaving the substrate blank wells empty, add 100 µl of diluted biotinylated antibody to all wells.

Cover plate(s) with adhesive seal, gently agitate the microtiter plate by tapping the edge of the plate for a few seconds to mix contents of each well and incubate for 1 hour at room temperature (18-25°C).

10. WASH STEP

Aspirate supernatant from wells and wash the microtiter plate(s) as described in point 6 above.

11. THIRD INCUBATION STEP **Streptavidin-HRP conjugate**

The kit contains one brown vial of concentrated streptavidin-HRP conjugate, which must be stored at -18°C to -32°C to maintain maximal stability. The contents of the vial will not be frozen at this temperature.

Per microtiter plate, add 12 µl streptavidin-HRP conjugate to 12 ml of working-strength dilution buffer just before use. Do not prepare in advance of assay.

Leaving the substrate blank wells empty, add 100 µl of streptavidin-HRP conjugate to all wells.

Cover the microtiter plate(s) with adhesive seal, agitate the microtiter plate by tapping the edge of the frame for a few seconds to mix contents of each well and incubate for 30 minutes at room temperature (18-25°C).

12. FOURTH WASH STEP

Aspirate supernatant from wells and wash the microtiter plate(s) as described in point 6 above.

13. FOURTH INCUBATION STEP **Enzymatic color development**

Approximately 10 minutes before use, prepare the substrate solution as described in this leaflet.

The substrate solution should be at room temperature (18-25°C) for optimal reproducible results.

Add 100 µl of substrate solution to all wells, **including the substrate blank wells**.

Cover microtiter plate(s) with lid, gently agitate the microtiter plate by tapping the edge of the frame for a few seconds to mix contents of each well and **incubate for 30 minutes at room temperature (18-25 °C) in the dark.** *Do not cover the plate with aluminum foil.*

Note: The speed of enzymatic color development is influenced by many factors including temperature and quality of the used TMB.

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14. STOP ENZYMATIC REACTION

Add 100 µl of stop solution to all wells.

After stopping the color is stable for maximally 30 minutes.

15. PLATE READ-OUT

Read at 450 nm in an ELISA reader

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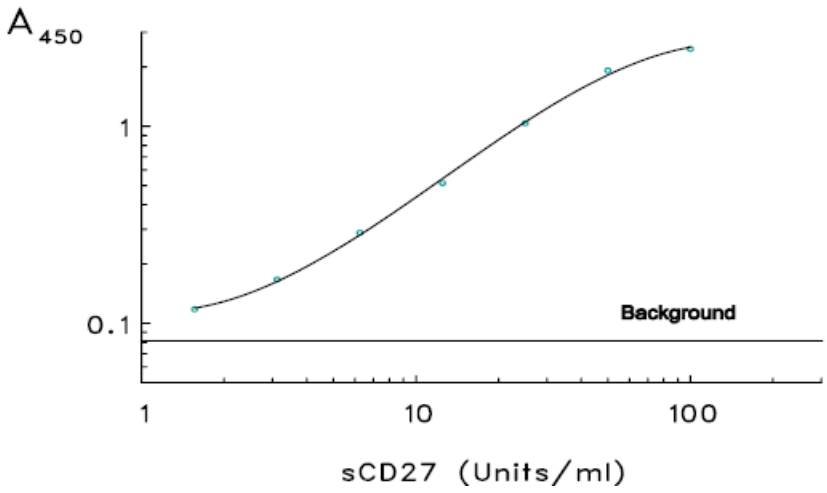
IX RESULTS

Standard curve

- Record the absorbance at 450 nm for each well containing standard, and average the duplicate values.
- Plot the average absorbances (ordinate) versus the sCD27 concentration in U/ml (abscissa) on log-linear paper and draw the best fitting curve. An example of a standard curve is given below.

A computer program to calculate ELISA results (developed by Mr. E.J. Nieuwenhuys, Sanquin Amsterdam) is available free of charge to our kit users.
This program can be downloaded from internet (see link at www.sanquinreagents.com)
http://www.xs4all.nl/~ednieuw/Logit/logit.htm



Typical standard curve for PeliKine compact™ sCD27 ELISA kit, data represent the MEAN of A_{450} nm readout (n=12).

Samples

- Record the absorbance at 450 nm for each standard well, and average the duplicate values.
- Locate the average absorbance value found for each sample on the vertical axis and follow a horizontal line intersecting the standard curve. At the point of intersection, read the sCD27 concentration (U/ml) from the horizontal axis. Multiply the obtained sCD27 concentration with the dilution factor of the sample and record this figure.

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Protocol summery and checklist PeliKine compact™ human sCD27 ELISA kit
Day 0:

o Bring coating antibody to room temperature (18-25°C).

o Prepare coating buffer.

o Dilute coating antibody 1:100 in coating buffer, add 100 µl to all wells, cover the plate(s) and incubate overnight at room temperature.

Day 1:

o Bring all reagents, with the exception of streptavidin-HRP, to room temperature.

o Prepare blocking buffer

o Wash the plate(s) five times with PBS.

o Add 200 µl dilution buffer to all wells and incubate for 30 minutes at room temperature.

o Prepare dilution buffer, standard and sample dilutions.

o Prepare washing buffer.

o Wash the plate(s) five times with washing buffer.

o Leaving the substrate blank wells empty, add 100 µl of standard and sample dilutions to the appropriate wells, cover the plate(s) and incubate for one hour at room temperature.

o Dilute biotinylated antibody 1:100 in dilution buffer.

o Wash the plate(s) five times with washing buffer.

o Leaving the substrate blank wells empty, add 100 µl of the diluted biotinylated antibody to all wells, cover the plate(s) and incubate for one hour at room temperature.

o Dilute the streptavidin-HRP conjugate 1:1,000 in dilution buffer.

o Wash the plate(s) five times with washing buffer.

o Leaving the substrate blank wells empty, add 100 µl of the streptavidin-HRP conjugate to all wells, cover plate(s) and incubate for 30 minutes at room temperature.

o Just before use, prepare substrate solution.

o Wash the plate(s) five times with washing buffer.

o Add 100 µl substrate solution to all wells, including the substrate blank wells, and incubate for 30 minutes at room temperature in the dark.

o Add 100 µl stop solution to all wells and read the plate at 450 nm.

o Calculate the amount of sCD27 in the samples.

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