# Quantikine® ELISA

## Human CD40 Ligand/TNFSF5 Immunoassay

Catalog Number DCDL40
SCDL40
PDCDL40

For the quantitative determination of human CD40 Ligand (CD40L) concentrations in cell culture supernates, serum, and platelet-poor plasma.

**Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.**

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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# INTRODUCTION

CD40 Ligand (CD40L), also known as CD154, gp39, TNFSF5, TRAP (TNF-Related Activation Protein) or TBAM (T-cell B-cell Activating Molecule), is a multifunctional ligand in the TNF superfamily (1-4). Interaction between CD40 and CD40L is critical to the control of thymus-dependent humoral immunity and cell-mediated immune responses (5-10). The major component of the contact-dependent signal leading to B cell activation is CD40L. CD40L stimulates B cell secretion of immunoglobulin isotypes in the presence of cytokines.

CD40L is a 39 kDa, 261 amino acid (aa) glycoprotein that can form homotrimers typical of other TNFSF members (1-4, 11, 12). Proteolytic cleavage can also produce 15-18 kDa soluble forms of CD40L (13, 14). Activated T cells and platelets express both a membrane-associated and a soluble form of CD40L (sCD40L) (13, 15, 16). Platelet activation during plasma and serum sample preparation can result in artificially elevated sCD40L levels (17-20). Conversely, serum samples stored above 2-8 °C show a progressive loss of the sCD40L signal (21). sCD40L lacks the transmembrane region and a portion of the extracellular domain but contains the entire TNF- homology region. Both the membrane-bound and soluble forms of CD40L are active (22).

The receptor for CD40L is CD40, a member of the TNF receptor superfamily (TNFRSF5). Interaction of CD40L with CD40 not only induces proliferation and isotype switching in B lymphocytes but also mediates a broad variety of other immune and inflammatory responses (5-7). CD40 signaling has been linked with pathogenic processes of chronic inflammatory diseases such as autoimmune diseases, neurodegenerative disorders, graft-versus-host disease, cancer, and atherosclerosis (8). The loss of interaction between CD40 and CD40L can result in impairment of T lymphocyte function, B lymphocyte differentiation, and monocyte function.

CD40L is expressed primarily on activated $CD4^+$ T cells; however, vascular endothelial cells, smooth muscle cells, macrophages, basophils, eosinophils, monocytes, dendritic cells, fibroblasts, and mast cells also express CD40L. Cytokine stimulation (e.g. IL-1β, TNF-α, or IFN-γ) can increase surface levels and *de novo* synthesis of CD40L in certain cell types (23). Hyper-IgM syndrome (HIGM) is an immunodeficiency characterized by elevated concentrations of serum IgM and the absence of serum IgG, IgA, and IgE. It is caused by mutations within the CD40L gene leading to defective expression on the membrane of activated T lymphocytes (24, 25). B lymphocytes from HIGM patients express functional CD40 and respond normally to wild-type CD40L, but their T lymphocytes are unable to stimulate CD40 signaling pathways (26, 27).

CD40L may play multiple roles in HIV infection (28). It may contribute to viral replication control by inducing HIV-suppressive chemokines, by downregulating monocyte cell surface expression of CCR5 and CD4, and by supporting the production of anti-HIV antibodies and cytotoxic T cells (28-31). It can also promote HIV replication in $CD4^+$ T lymphocytes by activating antigen-presenting cells, subsequently leading to increased $CD4^+$ T cell activation (28). With the onset of AIDS, CD40L-expressing $CD4^+$ T cells become selectively depleted. This loss may explain the similarity between the opportunistic infections characteristic of AIDS and those observed with congenital CD40L deficiency (28).

Elevated levels of sCD40L have been observed in sera from patients with systemic lupus erythematosus (SLE), chronic lymphocytic leukemia (CLL), and unstable angina (32-34). A direct relationship has been seen between disease severity and sCD40L in SLE patient sera (32). Aberrant expression of CD40L may thus contribute to autoantibody secretion in SLE through activation of bystander B lymphocytes, including cells that have been exposed to self antigens (32). Prolonged survival of malignant CLL cells may be linked to elevated levels of biologically active sCD40L (33). CD40L can mediate the resistance of CLL cells to apoptosis by Fas Ligand and fludarabine (33). Enhanced levels of both soluble and membrane-bound forms of CD40L in angina patients suggests that the CD40L-CD40 interaction may play a pathogenic role in the atherosclerotic process and in promoting acute coronary syndromes (34).

The Quantikine Human CD40 Ligand Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CD40 Ligand in cell culture supernates, serum, and platelet-poor plasma. It contains *E. coli*-expressed recombinant human CD40 Ligand and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CD40 Ligand showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human CD40 Ligand.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human CD40 Ligand has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD40 Ligand present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human CD40 Ligand is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CD40 Ligand bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | CATALOG # DCDL40 | CATALOG # SCDL40 | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human CD40 Ligand Microplate** | 890989 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human CD40 Ligand. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Human CD40 Ligand Standard** | 890991 | 1 vial | 6 vials | Recombinant human CD40 Ligand in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | May be stored for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles. |
| **Human CD40 Ligand Conjugate** | 890990 | 1 vial | 6 vials | 21 mL/vial of a polyclonal antibody specific for human CD40 Ligand conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-65** | 895527 | 1 vial | 6 vials | 12 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.* | |
| **Calibrator Diluent RD5P Concentrate** | 895151 | 1 vial | 6 vials | 21 mL/vial of a concentrated buffered protein base with preservatives. *Use diluted 1:2 in this assay.* | |
| **Wash Buffer Concentrate** | 895003 | 1 vial | 6 vials | 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL/vial of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

* Provided this is within the expiration date of the kit.

DCDL40 contains sufficient materials to run an ELISA on one 96 well plate.
SCDL40 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDCDL40). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human CD40 Ligand Controls (R&D Systems, Catalog # QC22).

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Because there may be species cross-reactivity in this kit, human CD40 Ligand levels in culture media containing equine serum cannot be assayed without interference.*

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Platelet-Poor Plasma** - Collect plasma on ice using heparin or EDTA as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

**CD40 Ligand is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of CD40 Ligand, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.**

## SAMPLE PREPARATION

Serum samples require at least a 5-fold dilution and may require up to a 20-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5P (diluted 1:2)*.

*See Reagent Preparation section.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

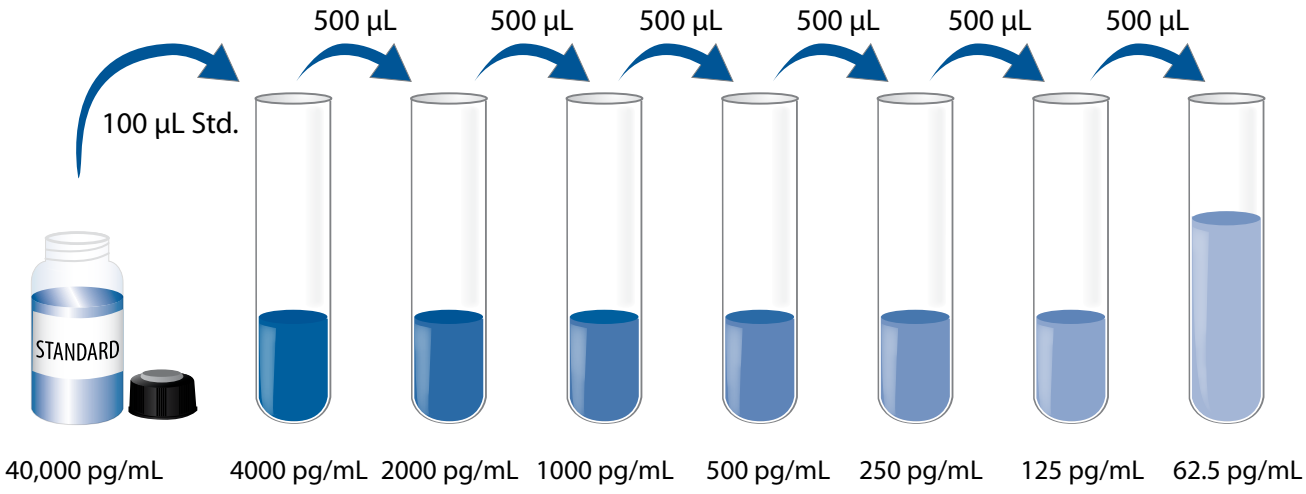
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Calibrator Diluent RD5P (diluted 1:2)** - Add 20 mL of Calibrator Diluent RD5P Concentrate to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5P (diluted 1:2).

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

**Human CD40 Ligand Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CD40 Ligand Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:2) into the 4000 pg/mL tube. Pipette 500 µL Calibrator Diluent RD5P (diluted 1:2) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:2) serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.**

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-65 to each well. Assay Diluent RD1-65 may contain undissolved material. Mix well before and during its use.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided as a record of samples and standards assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human CD40 Ligand Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours on the shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

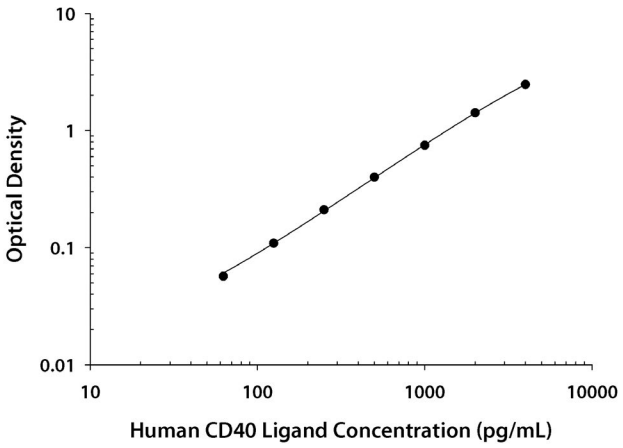
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human CD40 Ligand concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.039<br>0.039 | 0.039 | — |
| 62.5 | 0.096<br>0.096 | 0.096 | 0.057 |
| 125 | 0.146<br>0.150 | 0.148 | 0.109 |
| 250 | 0.250<br>0.250 | 0.250 | 0.211 |
| 500 | 0.439<br>0.439 | 0.439 | 0.400 |
| 1000 | 0.765<br>0.809 | 0.787 | 0.748 |
| 2000 | 1.439<br>1.469 | 1.454 | 1.415 |
| 4000 | 2.509<br>2.520 | 2.515 | 2.476 |

## PRECISION

**Intra-assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 40 | 40 | 40 |
| **Mean (pg/mL)** | 430 | 1212 | 2638 | 437 | 1205 | 2612 |
| **Standard deviation** | 21.8 | 54.4 | 143 | 27.9 | 72.7 | 162 |
| **CV (%)** | 5.1 | 4.5 | 5.4 | 6.4 | 6.0 | 6.2 |

## RECOVERY

The recovery of human CD40 Ligand spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 102 | 90-114% |
| Platelet-poor EDTA plasma (n=5) | 98 | 86-107% |
| Platelet-poor heparin plasma (n=5) | 94 | 86-113% |

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CD40 Ligand were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=4) | Serum* (n=7) | Platlet-poor EDTA plasma (n=5) | Platelet-poor heparin plasma (n=5) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 103 | 102 | 93 | 92 |
| | Range (%) | 99-105 | 96-109 | 89-97 | 87-97 |
| 1:4 | Average % of Expected | 105 | 106 | 102 | 102 |
| | Range (%) | 100-108 | 96-113 | 96-109 | 98-106 |
| 1:8 | Average % of Expected | 104 | 104 | 107 | 107 |
| | Range (%) | 102-109 | 95-111 | 99-115 | 103-109 |
| 1:16 | Average % of Expected | 104 | 94 | 101 | 105 |
| | Range (%) | 99-112 | 91-100 | 91-111 | 99-115 |

*Samples were diluted prior to assay as directed in the Sample Preparation section.

## SENSITIVITY

Thirty-nine assays were evaluated and the minimum detectable dose (MDD) of human CD40 Ligand ranged from 2.1-10.1 pg/mL. The mean MDD was 4.2 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human CD40 Ligand produced at R&D Systems.

## SAMPLE VALUES

**Serum/Plasma -** Samples from apparently healthy volunteers were evaluated for the presence of human CD40 Ligand in this assay. No medical histories were available for the donors used in this study.

| Sample Types | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Serum (n=58) | 5461 | 98 | ND-11,451 |
| Platelet-poor EDTA plasma (n=28) | 89.9 | 18 | ND-139 |
| Platelet-poor heparin plasma (n=22) | 89.8 | 4.5 | ND-89.8 |

ND=Non-detectable

**Cell Culture Supernates:**
Human peripheral blood mononuclear cells (1 x $10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum and 50 µM β-mercaptoethanol. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on day 1 and day 5 and assayed for levels of human CD40 Ligand.

| Condition | Day 1 (pg/mL) | Day 5 (pg/mL) |
|---|---|---|
| Unstimulated | ND | ND |
| Stimulated | 89.9 | 141 |

ND=Non-detectable

N1186 human T cells were cultured in RPMI with 10% fetal calf serum, 10 ng/mL rhIL-2, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 25 mM HEPES. The cells were stimulated for 48 hours with 10 mM PMA. An aliquot of the cell culture supernate was removed, assayed for human CD40 Ligand, and measured 102 pg/mL.

## SPECIFICITY

This assay recognizes natural and recombinant human CD40 Ligand.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human CD40 Ligand control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

ANG
ANG-2
AR
BDNF
CD4
CD40
CNTF
CT-1
CTLA-4
Epo
Fas
GDNF
GITR
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-12 p40
IL-13
IL-15
IL-16
IL-17
Leptin
LIF
MIF
NT-3
NT-4
OPG
OSM
PTN
SCF
SLPI
SMDF
TNF-α
TNF-β
Tpo
TRAIL
TRANCE

### Recombinant mouse:

CD40 Ligand
CT-1
CTLA-4
Fas
Fas Ligand
GITR Ligand
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12 p40
IL-13
IL-17
Leptin
LIF
OPG
OPN
OSM
SCF
TNF-α
Tpo
TRANCE

### Recombinant rat:

CNTF
GDNF
IFN-γ
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
Leptin
MK
TNF-α

### Recombinant porcine:

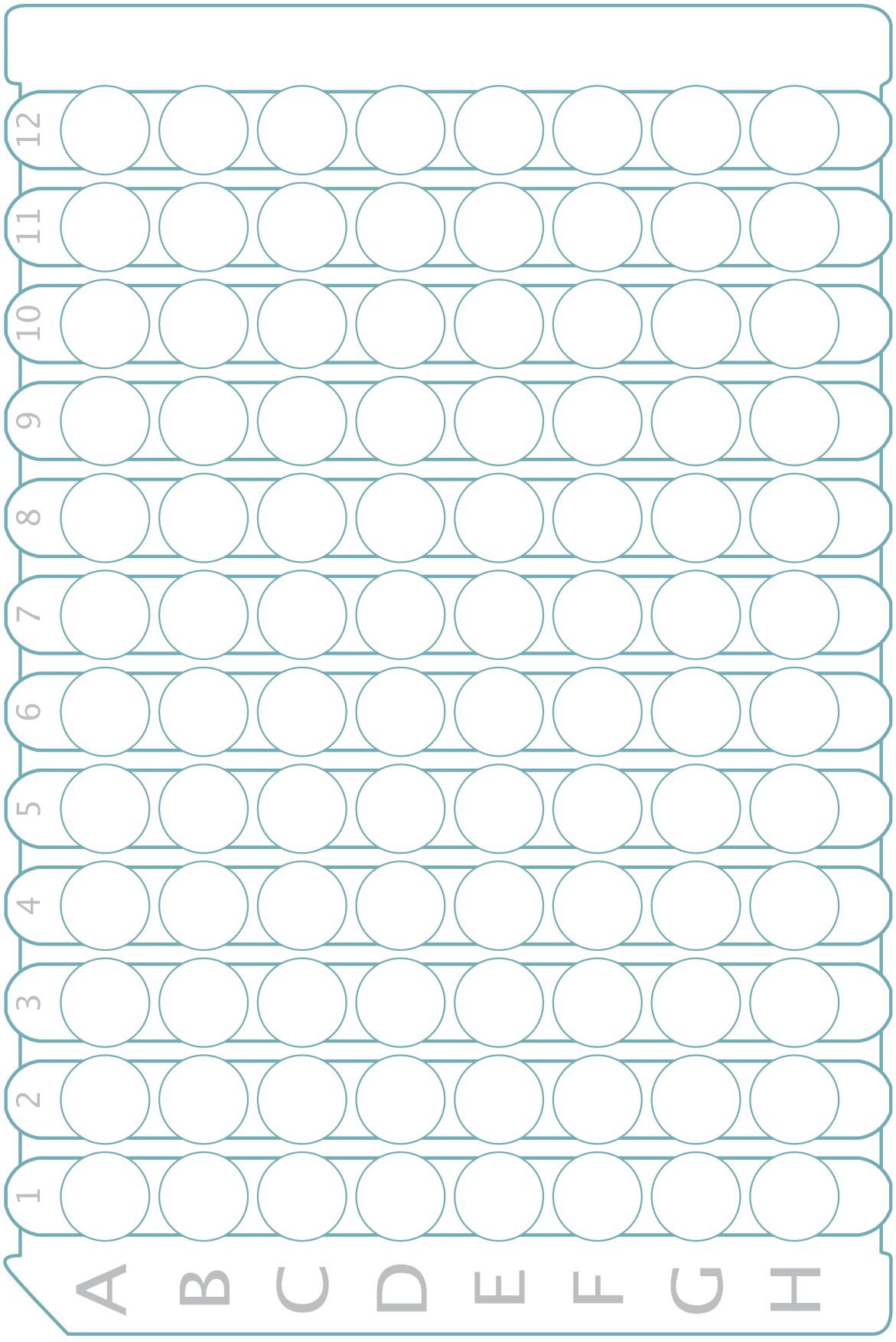
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-8
IL-10
TNF-α

## REFERENCES


1. Graf, D. *et al.* (1992) Eur. J. Immunol. **22**:3191.
2. Hollenbaugh, D. *et al.* (1992) EMBO J. **11**:4313.
3. Gauchat, J.F. *et al.* (1993) FEBS Lett. **315**:259.
4. Hermann, P. *et al.* (1993) Eur. J. Immunol. **23**:961.
5. Noelle, R.J. (1998) Agents Actions Suppl. **49**:17.
6. Grewal, I.S. and R.A. Flavell (1997) Immunol. Res. **16**:59.
7. Grewal, I.S. and R.A. Flavell (1998) Annu. Rev. Immunol. **16**:111.
8. Schonbeck, U. and P. Libby (2001) Cell Mol. Life Sci. **58**:4.
9. van Kooten, C. and J. Banchereau (2000) J. Leukoc. Biol. **67**:2.
10. Armitage, R.J. *et al.* (1993) Semin. Immunol. **5**:401.
11. Peitsch, M.C. and C.V. Jongeneel (1993) Int. Immunol. **5**:233.
12. Pietravalle, F. *et al.* (1996) J. Biol. Chem. **271**:5965.
13. Graf, D. *et al.* (1995) Eur. J. Immunol. **25**:1749.
14. Hsu, Y.M. *et al.* (1997) J. Biol. Chem. **272**:911.
15. Henn, V. *et al.* (2001) Blood **98**:1047.
16. Henn, V. *et al.* (1998) Nature **391**:591.
17. Weber, M. *et al.* (2006) Clin. Chem. **52**:888.
18. Khan, S.Y. *et al.* (2006) Blood **108**:2455.
19. Varo, N. *et al.* (2006) Clin. Sci. **111**:341.
20. Cognasse, F. *et al.* (2006) Transfusion **46**:1184.
21. Lengelle, J. *et al.* (2008) Cytokine **44**:275.
22. Mazzei, G.J. *et al.* (1995) J. Biol. Chem. **270**:7025.
23. Mach, F. *et al.* (1997) Proc. Natl. Acad. Sci. USA **94**:1931.
24. Callard, R.E. *et al.* (1993) Immunol. Today **14**:559.
25. Fuleihan, R.L. (1998) Semin. Hematol. **35**:321.
26. Aruffo, A. *et al.* (1993) Cell **72**:291.
27. Allen, R.C. *et al.* (1993) Science **259**:990.
28. Kornbluth, R.S. (2000) J. Leukoc. Biol. **68**:373.
29. Kornbluth, R.S. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:5205.
30. di Marzio, P. *et al.* (2000) Cytokine **12**:1489.
31. Ostrowski, M.A. *et al.* (2000) J. Immunol. **165**:6133.
32. Vakkalanka, R.K. *et al.* (1999) Arthritis Rheum. **42**:871.
33. Younes, A. *et al.* (1998) Br. J. Haematol. **100**:135.
34. Aukrust, P. *et al.* (1999) Circulation **100**:614.

## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



## NOTES