Instructions for Use

For the quantitative measurement of Human CD40 concentrations in cell culture supernatant, serum, plasma (EDTA, heparin) and amniotic fluid

This product is for research use only and is not intended for diagnostic use.
# Table of Contents

## INTRODUCTION
1. BACKGROUND 2
2. ASSAY SUMMARY 5

## GENERAL INFORMATION
3. PRECAUTIONS 6
4. STORAGE AND STABILITY 6
5. MATERIALS SUPPLIED 6
6. MATERIALS REQUIRED, NOT SUPPLIED 7
7. LIMITATIONS 7
8. TECHNICAL HINTS 8

## ASSAY PREPARATION
9. REAGENT PREPARATION 9
10. STANDARD PREPARATIONS 11
11. SAMPLE COLLECTION AND STORAGE 13
12. PLATE PREPARATION 14

## ASSAY PROCEDURE
13. ASSAY PROCEDURE 15

## DATA ANALYSIS
14. CALCULATIONS 18
15. TYPICAL DATA 19
16. TYPICAL SAMPLE VALUES 20
17. ASSAY SPECIFICITY 22

## RESOURCES
18. TROUBLESHOOTING 23
19. NOTES 24
INTRODUCTION

1. BACKGROUND

Abcam’s CD40 Human \textit{in vitro} ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate quantitative measurement of Human CD40 concentrations in cell culture supernatant, serum, plasma (EDTA, heparin) and amniotic fluid.

CD40 specific antibodies have been precoated onto 96-well plates. Standards and test samples are added to the wells along with a biotinylated CD40 detection antibody then incubated at room temperature and washed. A Streptavidin-HRP conjugate is then added to each well, incubated at room temperature and washed. TMB is added and catalyzed by HRP to produce a blue color product that changes into yellow after addition of acidic stop solution. The density of yellow coloration is directly proportional to the amount of CD40 sample captured in plate.

CD40 is a 50 kDa membrane-bound type I glycoprotein expressed by numerous cells, most notably B lymphocytes and monocytes and antigen presenting cells (APC) such as macrophages, dendritic cells and fibroblasts. It is furthermore highly expressed on various malignant cells. The expression of CD40 regulates T-cell - APC interaction and has been shown to be centrally involved in a wide array of inflammatory events. The function of CD40 is very broad.

CD40 belongs to the TNF-receptor family. The ligand for CD40 (CD154) is a 33 kDa type II transmembrane protein mainly expressed by activated T-cells, and is a member of the TNF superfamily. CD40/CD40L interactions are essential for T-cell-dependent B cell proliferation and differentiation, for activation of antigen presenting cells and for cytokine production on numerous other cells.

It is presently accepted that CD40 plays a critical role in the regulation of immune responses.
INTRODUCTION

CD40 expression has also been found on non lymphoid cells such as fibroblasts, endothelia, and epithelial cells.

Its critical role in T-cell-dependent humoral immune responses was demonstrated by patients with the hyper-IgM-Syndrome.

CD40 is also involved in the apoptotic pathway of cells. While it has been shown that the programmed cell death can be inhibited by the survival signals mediated from the binding of the CD40 receptor to the CD40 Ligand, very recently a novel proapoptotic mechanism induced by CD40 in carcinoma cells has been described. This mechanism is dependent on the endogenous production of cytotoxic cytokines.

The interaction of CD40 and its ligand, CD154 (CD40L) was found to play a crucial role in many aspects of immune response and the development and progress of various diseases.

It was found to be centrally involved in transplant rejection. The interaction of CD40 on synovial fibroblasts and CD40L expressed on activated T lymphocytes is directly involved in the neovascularization in rheumatoid synovitis. CD40 expression in thyroid tissue suggests a new pathway of pathogenesis of thyroid diseases. Functional expression of CD40 on Human melanoma cells mediates T-cell-co-stimulation and tumor cell growth.

The interaction of CD40 and CD154 is centrally involved in a wide array of inflammatory events such as multiple sclerosis, atherosclerosis and asthma-associated airway inflammation.

The expression of CD40 on various B cell malignancies such as leukemias, non Hodgkins lymphoma and multiple myeloma has been described. CD40 was found to regulate cell growth in squamous cell cancer of the head and neck. Its expression in hepatocellular carcinomas plays an important role in tumor biology.
INTRODUCTION

The expression of CD40 on Human lung cancer correlates with metastatic spread and may serve as a prognostic marker and an indicator of advanced disease.

An increased CD40 expression on muscle cells of polymyositis and dermatomyositis has been described.
2. ASSAY SUMMARY

Prepare all reagents, samples and standards as instructed.

Add standard or sample to each well used.

Add prepared detection antibody to each well. Incubate at room temperature.

Wash and add prepared Streptavidin-HRP conjugate. Incubate at room temperature.

Wash and add TMB Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.
3. **PRECAUTIONS**

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at 2-8°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9 Reagent Preparation.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate coated with anti-Human CD40 monoclonal antibody (12 x 8 wells)</td>
<td>96 wells</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Biotin-Conjugate anti-Human CD40 monoclonal antibody</td>
<td>100 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Streptavidin HRP</td>
<td>150 µL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>CD40 Standard Lyophilized (1000 pg/mL on reconstitution)</td>
<td>2 Vials</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>12 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>50 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>20X Assay Buffer Concentrate</td>
<td>5 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>TMB Substrate Solution</td>
<td>15 mL</td>
<td>2-8 ºC</td>
</tr>
<tr>
<td>Stop Solution (1 M Phosphoric Acid)</td>
<td>15 mL</td>
<td>2-8 ºC</td>
</tr>
</tbody>
</table>
6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 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)
- Microplate strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
8. **TECHNICAL HINTS**

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- As exact conditions may vary from assay to assay, a standard curve must be established for every run.
- 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 radio immunotherapy 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.
- **This kit is sold based on number of tests.** A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

9.1 1X Wash Buffer

Prepare 1X Wash Buffer by diluting the 20X Wash Buffer Concentrate with distilled or deionized water. To make 500 mL 1X Wash Buffer, combine 25 mL 20X Wash Buffer Concentrate with 475 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

*Note: The 1X Wash Buffer should be stored at 2-8 °C and is stable for 30 days.*

9.2 1X Assay Buffer

Prepare 1X Assay Buffer by diluting the 20X Assay Buffer Concentrate with distilled or deionized water. To make 50 mL 1X Assay Buffer, combine 2.5 mL 20X Assay Buffer Concentrate with 47.5 mL distilled or deionized water. Mix thoroughly and gently to avoid foaming.

*Note: The 1X Assay Buffer should be stored at 2-8 °C and is stable for 30 days.*
9.3 **1X Biotin Conjugated Antibody**

To prepare the Biotin Conjugated Antibody, dilute the anti-Human CD40 monoclonal antibody 100-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Biotin Conjugated Antibody as needed by adding the required volume (µL) of the Biotin Conjugated Antibody to the required volume (mL) of distilled water. Mix gently and thoroughly.

<table>
<thead>
<tr>
<th>Number of strips</th>
<th>Volume of Biotin-Conjugated CD40 antibody (µL)</th>
<th>1X Assay Buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>30</td>
<td>2.97</td>
</tr>
<tr>
<td>7 - 12</td>
<td>60</td>
<td>5.94</td>
</tr>
</tbody>
</table>

*Note: The 1X Biotin-Conjugated Antibody should be used within 30 minutes after dilution.*

9.4 **1X Streptavidin-HRP Conjugate**

To prepare the Streptavidin-HRP Conjugate, dilute the anti-Streptavidin-HRP Conjugate 100-fold with 1X Assay Buffer. Use the following table as a guide to prepare as much 1X Streptavidin-HRP Conjugate as needed by adding the required volume (µL) of the Streptavidin-HRP Conjugate to the required volume (mL) of distilled water. Mix gently and thoroughly.

<table>
<thead>
<tr>
<th>Number of strips</th>
<th>Volume of Streptavidin-HRP (µL)</th>
<th>1X Assay Buffer (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - 6</td>
<td>60</td>
<td>5.94</td>
</tr>
<tr>
<td>7 - 12</td>
<td>120</td>
<td>11.88</td>
</tr>
</tbody>
</table>

*Note: The 1X Streptavidin-HRP should be used within 30 minutes after dilution.*

- All other solutions are supplied ready to use
10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Prepare a 1,000 pg/mL Stock Standard by reconstituting one vial of the Human CD40 standard with the volume of distilled water stated on the label. Hold at room temperature for 10-30 minutes. The 1,000 ng/mL Stock Standard cannot be stored for later use.

10.2 Label eight tubes with numbers 1 - 8.

10.3 Add 225 µL sample diluent to all tubes.

10.4 Prepare a 500 ng/mL Standard 1 by adding 225 µL of the 1000 ng/mL Stock Standard to tube 1. Mix thoroughly and gently.

10.5 Prepare Standard 2 by transferring 225 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare Standard 3 by transferring 225 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes number 4 through to 7.

10.8 Standard 8 contains no protein and is the Blank control.
## ASSAY PREPARATION

<table>
<thead>
<tr>
<th>Standard</th>
<th>Sample to Dilute</th>
<th>Volume to Dilute (µL)</th>
<th>Volume of Diluent (µL)</th>
<th>Starting Conc. (pg/mL)</th>
<th>Final Conc. (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>225</td>
<td>225</td>
<td>1,000</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>Standard 1</td>
<td>225</td>
<td>225</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>Standard 2</td>
<td>225</td>
<td>225</td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>4</td>
<td>Standard 3</td>
<td>225</td>
<td>225</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>5</td>
<td>Standard 4</td>
<td>225</td>
<td>225</td>
<td>62.5</td>
<td>31.3</td>
</tr>
<tr>
<td>6</td>
<td>Standard 5</td>
<td>225</td>
<td>225</td>
<td>31.3</td>
<td>15.6</td>
</tr>
<tr>
<td>7</td>
<td>Standard 6</td>
<td>225</td>
<td>225</td>
<td>15.6</td>
<td>7.8</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>-</td>
<td>225</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

![Diagram of assay preparation process]
11. SAMPLE COLLECTION AND STORAGE

- Cell culture supernatant, serum, plasma (EDTA, heparin) and amniotic fluid were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum from the clot or cells 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 aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Human CD40. If samples are to be run within 24 hours, they may be stored at 2° to 8°C.

- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently and properly diluted with 1X Sample Diluent.

- Aliquots of serum samples (spiked or unspiked) were stored at -20°C and thawed several times, and the Human CD40 levels determined. There was no significant loss of Human CD40 immunoreactivity detected by freezing and thawing.
12. **PLATE PREPARATION**

- The 96 well plate strips included with this kit are supplied ready to use
- Unused well strips should be returned to the plate packet and stored at 2-8°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay
13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections. Determine the number of microplate strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards.

13.2. Wash the microplate twice with approximately 400 µL 1X Wash Buffer per well with thorough aspiration of microplate contents between washes. Allow the 1X Wash Buffer to remain in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microplate.

13.3. After the last wash step, empty wells and tap microplate on absorbent pad or paper towel to remove excess 1X Wash Buffer. Use the microplate strips immediately after washing. Alternatively the microplate strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

13.4. Add 100 µL of prepared standards (including the standard blank control) to the appropriate wells.

13.5. Add 50 µL of sample to appropriate wells.

13.6. Add 50 µL of Sample Diluent to all sample wells.

13.7. Add 50 µL of 1X Biotin Conjugated Antibody to all wells.

13.8. Cover with adhesive film and incubate at room temperature (18° to 25°C) for 2 hours (microplate can be incubated on a shaker set at 400 rpm).

13.9. Remove adhesive film and empty wells. Wash microplate strips 6 times according to step 13.2. Proceed immediately to step 13.10.
13.10. Add 100 µL of 1X Streptavidin-HRP to all wells, including the blank wells.

13.11. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour (microplate can be incubated on a shaker set at 100 rpm).

13.12. Remove adhesive film and empty wells. Wash microplate strips 3 times according to step 13.2. Proceed immediately to the next step.

13.13. Pipette 100 µL of TMB Substrate Solution to all wells.

13.14. Incubate the microplate strips at room temperature (18 to 25°C) for 10 minutes. Avoid direct exposure to intense light.

*Note:* The color development on the plate should be monitored and the substrate reaction stopped (see step 13.15) before the signal in the positive wells becomes saturated. Determination of the ideal time period for color development should to be done individually for each assay. 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 Standard 1 has reached an OD of 0.9 - 0.95.

13.15. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well.

*Note:* It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microplate strips are stored at 2 - 8°C in the dark.

13.16. Read absorbance of each microplate on a spectrophotometer 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 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.
14. DATA ANALYSIS

**CALCULATIONS**

Average the duplicate readings for each standard, sample and control blank. Subtract the no protein control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A five parameter algorithm (5PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 5-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

If instructions in this protocol have been followed samples have been diluted 1:2, as stated in Step 13.6, the concentration read from the standard curve must be multiplied by the dilution factor (x 2), to obtain an accurate value, in addition to any initial sample dilution factor.

Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low Human CD40 levels (Hook Effect). Such samples require further external predilution according to expected Human CD40 values with Sample Diluent in order to precisely quantitate the actual Human CD40 level.
15. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for *demonstration purposes only*. A new standard curve must be generated for each assay performed.

![Typical Standard Curve](image)

<table>
<thead>
<tr>
<th>Conc. (pg/mL)</th>
<th>O.D. 450 nm</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.024</td>
<td>0.028</td>
</tr>
<tr>
<td>7.8</td>
<td>0.122</td>
<td>0.122</td>
</tr>
<tr>
<td>15.6</td>
<td>0.197</td>
<td>0.193</td>
</tr>
<tr>
<td>31.3</td>
<td>0.345</td>
<td>0.354</td>
</tr>
<tr>
<td>62.5</td>
<td>0.604</td>
<td>0.610</td>
</tr>
<tr>
<td>125</td>
<td>1.069</td>
<td>1.052</td>
</tr>
<tr>
<td>250</td>
<td>1.727</td>
<td>1.711</td>
</tr>
<tr>
<td>500</td>
<td>2.325</td>
<td>2.330</td>
</tr>
</tbody>
</table>

**Figure 1.** Example of Human and the Human CD40 standard protein standard curve.
16. TYPICAL SAMPLE VALUES

SENSITIVITY –
The limit of detection for CD40 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 1.3 pg/mL (mean of 6 independent assays).

RECOVERY –
Spiked samples were prepared by adding four different levels of recombinant CD40 into serum. Recoveries were determined in 3 independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. The recovery ranged from 81% to 110% with an overall mean recovery of 94%.

LINEARITY OF DILUTION –
A serum sample was assayed at four two-fold dilutions covering the working range of the standard curve. Recoveries ranged from 82% to 104% with an overall mean recovery of 94%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>CD40 Expected Concentration (pg/mL)</th>
<th>CD40 Observed Concentration (pg/mL)</th>
<th>Recovery of Expected Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:2</td>
<td>-</td>
<td>121.4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>60.7</td>
<td>62.9</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>30.3</td>
<td>31.4</td>
<td>104</td>
</tr>
<tr>
<td>2</td>
<td>1:2</td>
<td>-</td>
<td>88.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>44.0</td>
<td>43.2</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>22.0</td>
<td>21.4</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>1:2</td>
<td>-</td>
<td>419.9</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>209.9</td>
<td>197.2</td>
<td>94</td>
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<tr>
<td></td>
<td>1:8</td>
<td>105.0</td>
<td>85.7</td>
<td>82</td>
</tr>
<tr>
<td>4</td>
<td>1:2</td>
<td>-</td>
<td>282.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>141.0</td>
<td>130.7</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>1:8</td>
<td>70.5</td>
<td>58.8</td>
<td>83</td>
</tr>
</tbody>
</table>
COMPARISON OF SERUM AND PLASMA –
Serum as well as EDTA, citrate and heparin plasma were obtained from 8 individuals were evaluated. Human sCD40 concentrations were not significantly different and therefore all these body fluids are suitable for the assay. It is nevertheless highly recommended to assure the uniformity of blood preparations.

PRECISION –
Intra- and Inter-assay reproducibility was determined by measuring samples containing different concentrations of Human CD137.

<table>
<thead>
<tr>
<th></th>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>%CV</td>
<td>5.5</td>
<td>7.0</td>
</tr>
</tbody>
</table>

EXPECTED VALUES –
A panel of 8 serum samples from randomly selected apparently healthy donors was tested for Human CD40.

The detected Human CD40 levels ranged between 53.3 and 156.9 pg/mL with a mean level of 102.1 pg/mL and a standard deviation of 37.1 pg/mL.
17. ASSAY SPECIFICITY
The assay detects both endogenous and recombinant Human CD40.

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a CD40 positive serum. There was no detectable cross reactivity with any of the tested proteins.
# 18. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
<td></td>
</tr>
<tr>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
<td></td>
</tr>
<tr>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
<td></td>
</tr>
<tr>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
<td></td>
</tr>
<tr>
<td>Starting sample concentration is too high.</td>
<td>Dilute the specimens and repeat the assay</td>
<td></td>
</tr>
<tr>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
<td></td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Improper storage of the kit</td>
<td>Store the all components as directed.</td>
<td></td>
</tr>
</tbody>
</table>
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國联通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

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