Speedy™ Mouse CD40 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE60035

Size: 96T

Sensitivity: 2.5 pg/mL

Range: 31.25-2000 pg/mL, 15.6-1000 pg/mL

Usage: For the quantitative detection of mouse CD40 concentrations in serum, plasma, cell culture supernatant and tissue lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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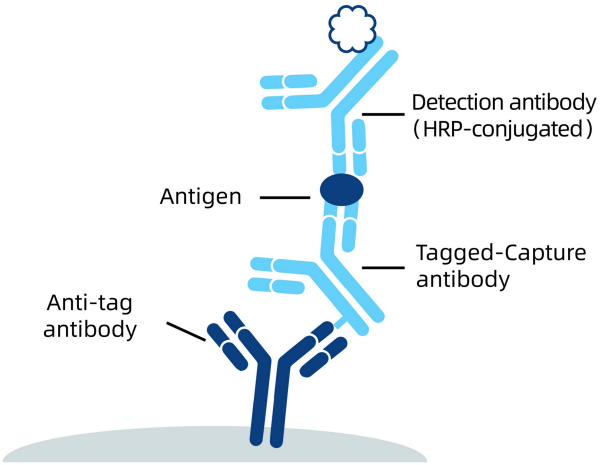
1. Background

CD40, also named as TNFRSF5 and Bp50, is a type I transmembrane protein that belongs to the tumor necrosis factor receptor (TNFR) family. CD40 is expressed on B cells, dendritic cells (DCs), monocytes, platelets, and macrophages as well as by non-hematopoietic cells such as myofibroblasts, fibroblasts, epithelial, and endothelial cells. It is a receptor for TNFSF5/CD40L. CD40L/CD40 interactions are essential for immunoglobulin (Ig) isotype switching, germinal center formation and the development of B cell memory.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the solution yellow. The color intensity is proportional to the quantity of bound protein, which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended. Proteintech data analysis website, https: //www.ptgcn.com/products/elisa-kits/.

3.8 Microplate thermostatic shaker.

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4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 2000 pg/bottle; lyophilized	2 bottles	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Additional Diluent AT-60035 - 6 mL/bottle. Only for serum and plasma samples	1 bottle	
Sample Diluent PT 1 - 30 mL/bottle. For mouse serum and plasma.	1 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle. For cell culture supernatant and tissue lysate.	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 15 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

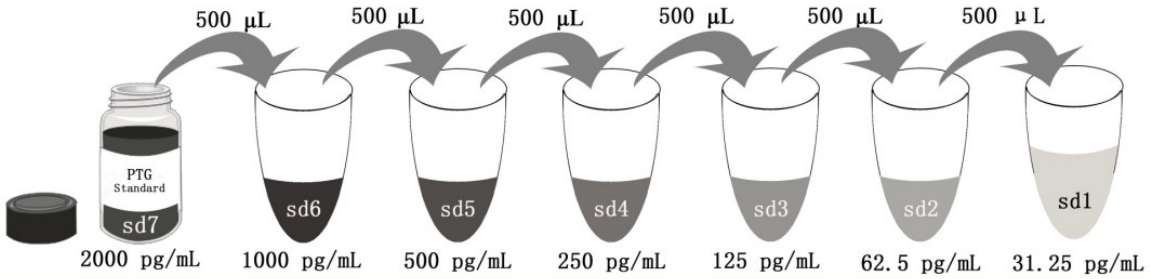
7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement .

Recommended Dilution for different sample types: 1:2 is recommended for mouse serum and plasma; 1:2 to 1:8 is recommended for cell culture supernatant; 1:2 to 1:160 is recommended for tissue lysate.

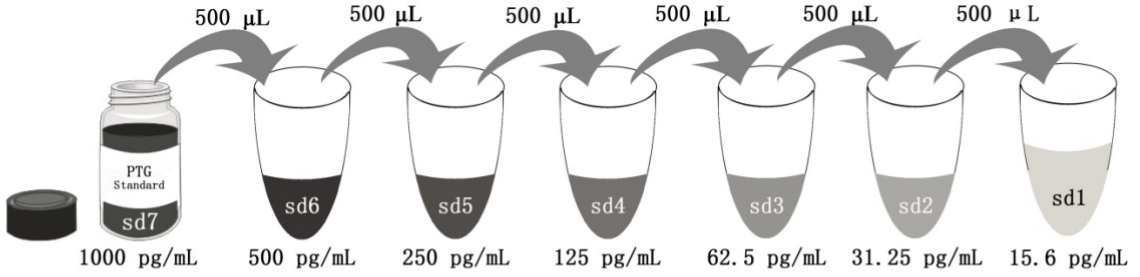
7.4 Standard Serial Dilution:

For mouse serum and plasma, add 1 mL Sample Diluent PT 1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

For cell culture supernatant and tissue lysate, add 2 mL Sample Diluent PT 4B1 in protein standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated antibody can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group;

For serum or plasma, add 25 µL of Additional Diluent to the appropriate wells (No need incubation and wash);

For cell culture supernatant and tissue lysate, no need to add Additional Diluent, directly follow the next step.

8.3 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.4 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

8.5 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1× Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.6 Add 100 µL TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.7 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.8 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.9 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, four-parameter logistic curve- fit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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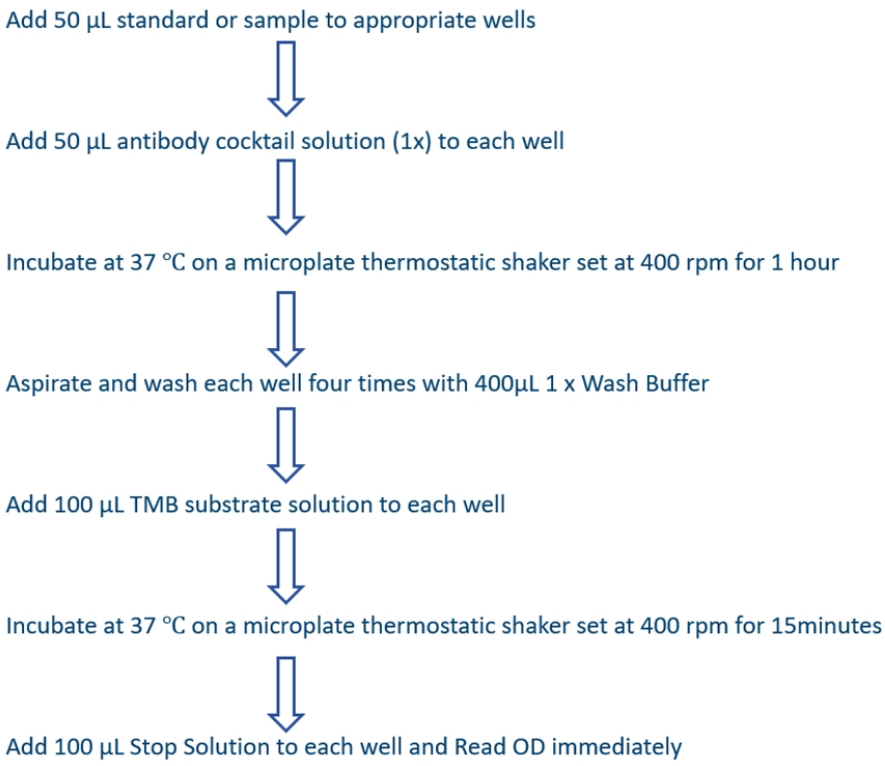

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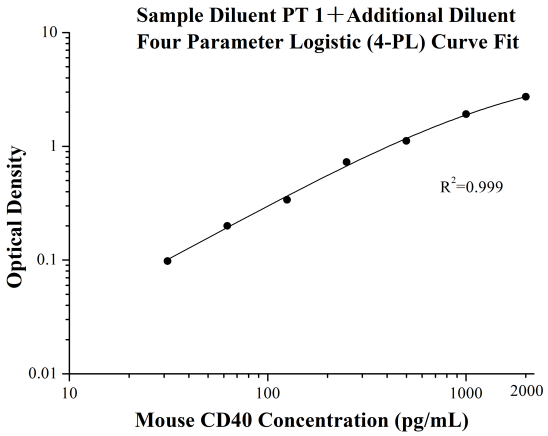
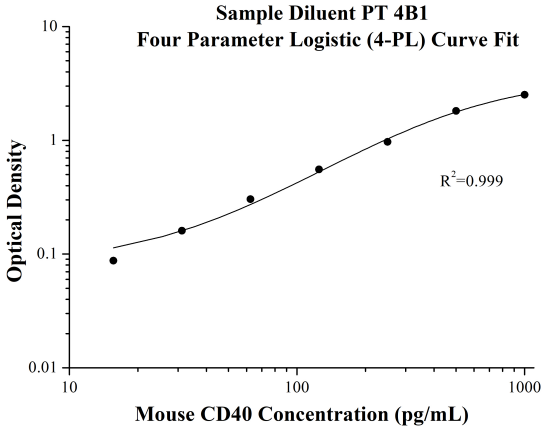
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.0296 0.0272	0.0284	-
31.25	0.1215 0.1308	0.12615	0.09775
62.5	0.2302 0.2264	0.2283	0.1999
125	0.361 0.3748	0.3679	0.3395
250	0.7433 0.7706	0.75695	0.72855
500	1.1367 1.1543	1.1455	1.1171
1000	1.9328 1.9541	1.94345	1.91505
2000	2.8003 2.7125	2.7564	2.728



(pg/mL)	O.D	Average	Corrected
0	0.0431 0.0441	0.0436	-
15.6	0.1189 0.1435	0.1312	0.0876
31.25	0.2006 0.2066	0.2036	0.16
62.5	0.3343 0.3613	0.3478	0.3042
125	0.6012 0.5963	0.59875	0.55515
250	0.9956 1.0305	1.01305	0.96945
500	1.9837 1.7272	1.85545	1.81185
1000	2.5486 2.5743	2.56145	2.51785

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9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	650.6	31.5	4.8
2	8	165.6	3.6	2.2
3	8	87.5	3.3	3.8

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	623.4	37.1	6.0
2	16	159.1	7.6	4.8
3	16	88.5	6.1	6.9

9.3 Recovery

The recovery of mouse CD40 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:2	91	83-98
	1:4	117	112-125
Cell culture supernatant	1:2	92	88-98
	1:4	81	75-86
Tissue lysate	1:4	93	86-99
	1:8	84	81-87

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9.4 Sample values

Mouse serum - mouse serum samples were evaluated for the presence of mouse CD40 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse serum (n=16)	156.8	63.7-618.8

Cell culture supernatant - Mouse spleens were homogenized using a tissue homogenizer. Cells (1 x 10^6 cells/ mL) were cultured unstimulated or stimulated with 1 µg/mL of LPS for 3 days in RPMI supplemented with 10% fetal bovine serum. Aliquots of the cell culture supernates were removed, assayed for levels of mouse CD40, and measured less than the lowest standard, 15.6 pg/mL.

Mouse kidneys were homogenized using a tissue homogenizer. Cells (1 x 10^6 cells/ mL) were cultured unstimulated or stimulated with 1 µg/mL of LPS for 3 days in RPMI supplemented with 10% fetal bovine serum. Aliquots of the cell culture supernates were removed, assayed for levels of mouse CD40, and measured less than the lowest standard, 15.6 pg/mL.

Mouse lungs were homogenized using a tissue homogenizer. Cells (1 x 10^6 cells/ mL) were cultured unstimulated or stimulated with 5 µg/mL of LPS for 2 days in RPMI supplemented with 10% fetal bovine serum. Aliquots of the cell culture supernates were removed and assayed for levels of mouse CD40.

Condition	Day 2 (pg/mL)
Unstimulated	ND
Stimulated	491.7

ND*=Non-detectable

J774A.1 (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 5µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS and 20 ng/mL IFNγ for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of mouse CD40.

Condition	Day 1 (pg/mL)
Unstimulated	85.3
Stimulated	1,549.9

Tissue lysate

	Mouse CD40 (**ng/mL**)	Total protein (**mg/mL**)
Mouse spleen tissue lysate	52.1	4.8
Mouse heart tissue lysate	1.1	6.9
Mouse kidney tissue lysate	0.3	3.1
Mouse brain tissue lysate	0.2	7.0
Mouse lung tissue lysate	22.7	5.3

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9.5 Sensitivity

The minimum detectable dose of mouse CD40 is 2.5 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, mouse serum samples were spiked with high concentrations of mouse CD40 and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell culture surpernatant and cell lysate samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The cell lysate was initially diluted 1:40.)

		Mouse serum (Sample Diluent PT 1)	Cell culture supernatant (Sample Diluent PT 4B1)	Cell lysate (Sample Diluent PT 4B1)
1:2	Average% of Expected	90	100	100
	Range (%)	79-101	-	-
1:4	Average% of Expected	121	102	105
	Range (%)	112-130	99-106	104-106
1:8	Average% of Expected	121	91	108
	Range (%)	121-122	89-94	103-112
1:16	Average% of Expected	110	98	110
	Range (%)	105-115	97-99	105-116

9.7 Specificity

This assay recognizes natural and recombinant mouse CD40.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

CD40

Recombinant mouse:

CD40 Ligand

Fas

10. References

1. Foy, T M et al. The Journal of experimental medicine vol. 180,1 (1994): 157-63.

2. van Kooten, C, and J Banchereau. Journal of leukocyte biology vol. 67,1 (2000): 2-17.

3. Ferrari, S et al. Proceedings of the National Academy of Sciences of the United States of America vol. 98,22 (2001): 12614-9.

4. Elgueta, Raul et al. Immunological reviews vol. 229,1 (2009): 152-72.

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