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Sunlong Medical™ Human CTLA-4/CD152 One-Step ELISA Kit

Catalog Number: One-EL080Hu
Size: 96 Test

Storage: 2-8℃/-20℃
Validity Period: 2-8℃: 6 months; -20℃: 12 months

Sensitivity: 7.8 pg/mL
Assay range: 23.4-1500 pg/mL

For the quantitative detection of human CTLA-4/CD152 concentrations in serum, plasma, cell culture supernatant, urine

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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ASSAY PROCEDURE SUMMARY

1. Preset the layout of the microplate, including control group, standard group and sample group, 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.

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2. 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.

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3. 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).

↓

4. 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.

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5. 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.)

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6. 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.

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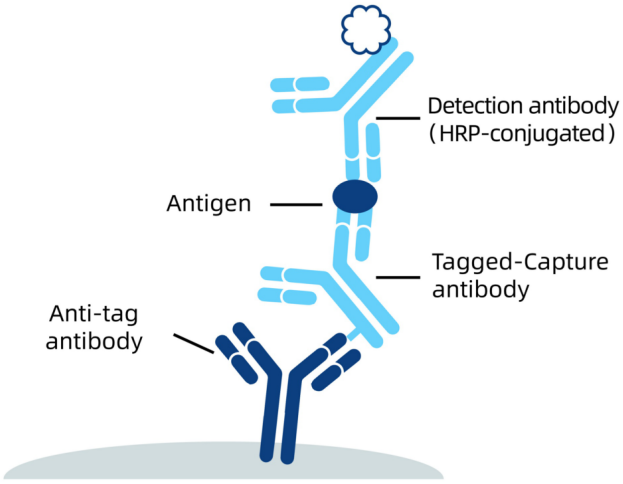
7. Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

DESCRIPTION

CTLA-4, also known as CD152, belonging to the immunoglobulin superfamily, is primarily found on activated T cells and regulatory T cells (Tregs). CTLA-4 is closely related to the T-cell costimulatory CD28, and both molecules bind to B7-1 and B7-2 on antigen-presenting cells. CTLA-4 acts as a negative regulatory molecule of T-cell responses. Besides the full-length transmembrane form, CTLA-4 also exists in a truncated soluble form (sCTLA-4).

PRINCIPLE OF THE ASSAY

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.



LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use expired kit or reagents.
- Do not mix or substitute reagents or materials from other kit lots or other sources.
- If concentration of assayed factor in samples is higher than the highest standard, dilute the samples with the appropriate Sample Diluent and reanalyze.
- Any variation in testing personnel, sample preparation, standard dilution, pipetting technique, washing techniques, incubation time, temperature, kit age and equipment can cause variation in results.
- This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED (96 Test)

Unopened kit should be stored at 2 - 8°C.

- **Microplate:** 96 well microplate precoated an anti-tag antibody (8 well × 12 strips) (1 plate)
- **Protein standard:** 3000 pg/bottle; lyophilized (2 bottles)
- **Capture antibody (100×):** 60 µL/vial* (1 vial)
- **Detection antibody, HRP-conjugated (100×):** 60 µL/vial* (1 vial)
- **Sample Diluent PT 1:** 30 mL/bottle. For human serum and plasma. (1 bottle)
- **Sample Diluent PT 4B1:** 30 mL/bottle. For cell culture supernatant, urine and cell 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)

STORAGE

Store kit reagents between 2 and 8℃. Immediately after use remaining reagents should be returned to cold storage (2 to 8℃). Expiration date of the kit and reagents is stated on the labels.

Expiration date of the kit components can only be guaranteed if the components are stored properly.

	Condition
Unopened kit	Store at 2 - 8℃ for 6 months or -20℃ for 12 months. (See expiration date on the label.)
Opened kit	All reagents stored at 2 - 8℃ for 7 days. Please use a new standard for each assay.

OTHER SUPPLIES REQUIRED

- A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- Plate washer: automated or manual.
- Absorbent paper towels.
- Glass or plastic tubes to prepare standard and sample dilutions.
- Beakers and graduated cylinders.
- 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.
- Microplate thermostatic shaker.

PRECAUTION

- Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- Do not use the kit after the expiration date.
- Do not mix or substitute reagents or materials from other kit lots or other sources.
- Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

TECHNICAL HINTS

- Bring all reagents to room temperature before use.
- 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.
- When using an automated plate washer, adding a 30 second soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- It is recommended that all samples and standards be assayed in duplicate.

SAMPLE COLLECTION AND STORAGE

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℃. Avoid repeated freeze-thaw cycles.

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℃. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatant

Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Urine

Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

Cell Lysate

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times. 2) Count cells and then discard the supernatant. 3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis. 4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 107 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples. 5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4℃. 6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use. If crystals have formed in the Wash Buffer Concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

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.

Antibody Cocktail (1X)

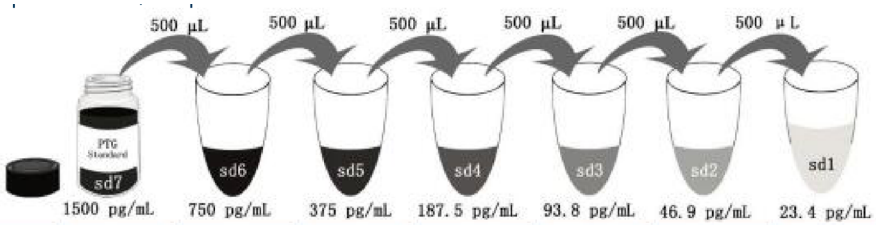
Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

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 human serum, plasma, cell culture supernatant, urine and cell lysate.

Standard Serial Dilution

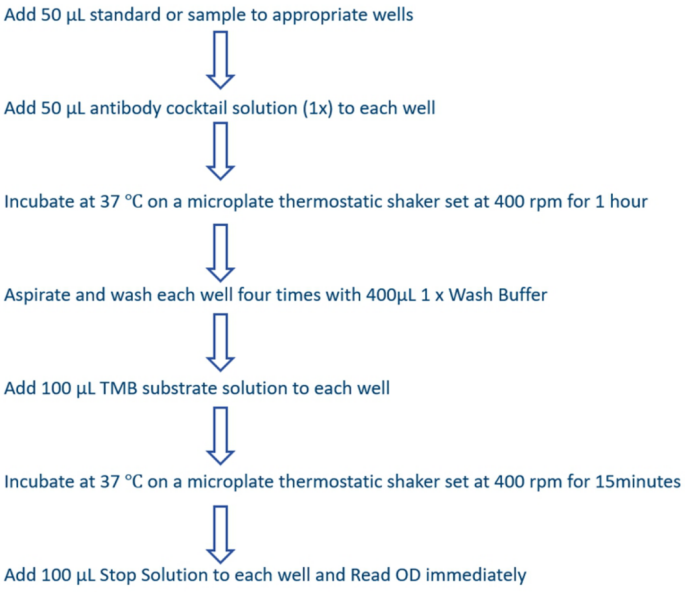
For human serum and plasma, add 2 mL Sample Diluent PT 1 in protein standard. For cell culture supernatant, urine and cell lysate, add 2 mL Sample Diluent PT 4B1 in protein standard.



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 1 or PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

ASSAY PROCEDURE

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.



1. Preset the layout of the microplate, including control group, standard group and sample group, 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.

2. 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.

3. 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).

4. 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.

5. 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.)

6. 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.

7. 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).

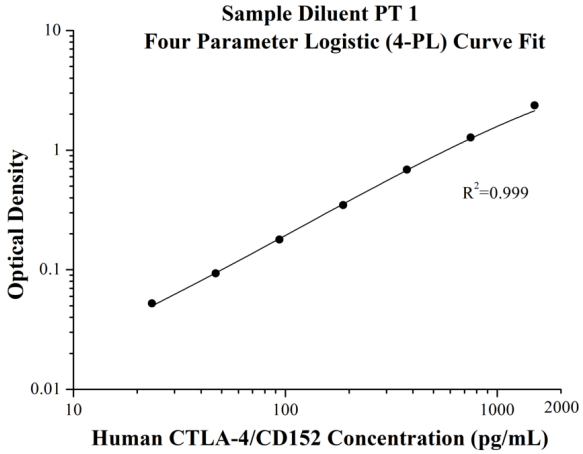
CALCULATION OF RESULTS

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 curvefit (4-PL) analysis is recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

TYPICAL DATA

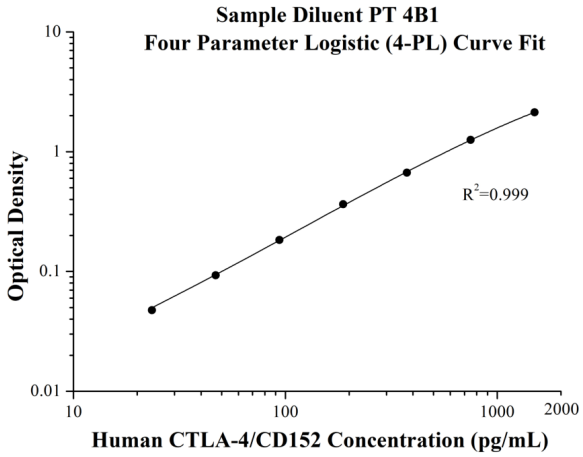
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard curve 1



(pg/mL)	O.D.	Average	Corrected
0	0.0152 0.0131	0.01415	-
23.4	0.0704 0.0627	0.06655	0.0524
46.9	0.1095 0.1052	0.10735	0.0932
93.8	0.1971 0.1891	0.1931	0.17895
187.5	0.3705 0.3527	0.3616	0.34745
375	0.6603 0.7413	0.7008	0.68665
750	1.3589 1.2246	1.29175	1.2776
1500	2.3786 2.3862	2.3824	2.36825

Standard curve 2



(pg/mL)	O.D.	Average	Corrected
0	0.0234 0.0181	0.02075	-
23.4	0.0724 0.064	0.0682	0.04745
46.9	0.1198 0.1076	0.1137	0.09295
93.8	0.2152 0.193	0.2041	0.18335
187.5	0.4079 0.3626	0.38525	0.3645
375	0.7051 0.6717	0.6884	0.66765
750	1.3652 1.1913	1.27825	1.2575
1500	2.2009 2.1075	2.1542	2.13345

SENSITIVITY

The minimum detectable dose of human CTLA-4/CD152 is 7.8 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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	725.7	23.3	3.2
2	8	184.8	4.0	2.2
3	8	101.8	3.0	2.9

Inter-assay Precision

Sample	n	Mean (pg/mL)	SD	CV%
1	16	703.0	55.2	7.9
2	16	175.6	10.2	5.8
3	16	98.2	4.6	4.7

RECOVERY

The recovery of human CTLA-4/CD152 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:2	82	79-85
	1:4	107	103-109
Cell culture supernatant	1:2	87	79-92
	1:4	89	80-97
Urine	1:2	75	70-80
	1:4	91	84-100
Cell lysate	1:2	83	72-92
	1:4	94	90-96

LINEARITY

To assess the linearity of the assay, human plasma, urine and cell lysate samples were spiked with high concenthumanions of human CTLA-4/CD152 and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Cell culture supernatant samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma (Sample Diluent PT 1)	Cell culture supernatant (Sample Diluent PT 4B1)	Urine (Sample Diluent PT 4B1)	Cell lysate (Sample Diluent PT 4B1)
1:2	Average% of Expected	83	100	78	71
	Range (%)	81-85	-	76-79	70-73
1:4	Average% of Expected	107	87	89	78
	Range (%)	105-109	86-88	88-90	78-79
1:8	Average% of Expected	108	89	96	85
	Range (%)	101-114	88-91	96-97	84-86
1:16	Average% of Expected	101	-	101	89
	Range (%)	90-112	-	99-102	88-91

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant **human Human CTLA-4/CD152 One-Step ELISA Kit** produced at Sunlong Medical™.

SAMPLE VALUES

Human plasma - Sixteen samples were evaluated for detectable levels of human CTLA-4/CD152 in this assay. Thirteen measured less than the lowest human CTLA-4/CD152 standard, 23.4 pg/mL. Three samples read 59.7 pg/mL, 70.7 pg/mL and 42.7 pg/mL.

Cell culture supernatant - Human peripheral blood mononuclear cells (PBMC) (1 x 106 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 10 µg/mL PHA for 1 day, 3 days and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human CTLA-4/CD152. ND*=Non-detectable

Urine - Eight urine samples from volunteers were evaluated for human CTLA-4/CD152 in this assay. All samples measured less than the lowest standard, 23.4 pg/mL. No medical histories were available for the donors used in this study.

Cell lysate - PBMC cell lysate and Jurkat cell lysate samples were evaluated for human CTLA-4/CD152 in this assay. All samples measured less than the lowest standard, 23.4 pg/mL.

Condition	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND	ND
Stimulated	ND	108.3	114.5

SPECIFICITY

This assay recognizes natural and recombinant human CTLA-4/CD152. The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference. Recombinant human: Recombinant mouse: B7-1 CTLA-4 B7-2 CD28 IL-17

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												