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# Sunlong Medical™ Human CTLA-4/CD152 Sandwich ELISA Kit

**Catalog Number:** EL0555Hu
**Size:** 96 Test

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

**Sensitivity:** 3.7 pg/mL
**Assay range:** 23.4-1500 pg/mL

For the quantitative detection of human CTLA-4/CD152 concentrations in serum, plasma, urine and cell lysate.

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. 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. Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).**

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**3. Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.**

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**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 1X 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 of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.**

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**6. Repeat wash step in 8.4.**

↓

**7. Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.**

↓

**8. Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.**

↓

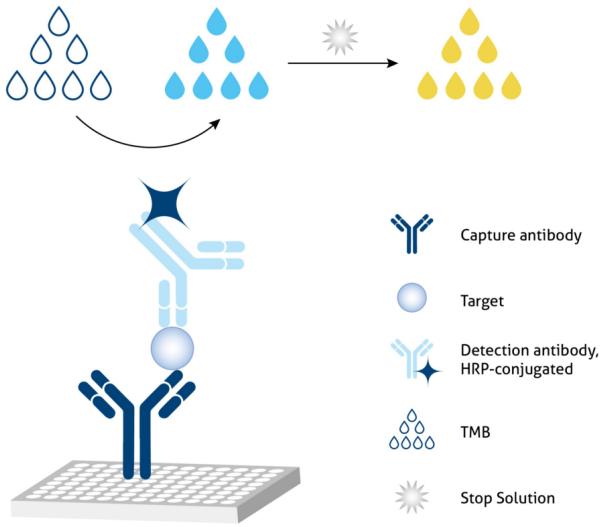
**9. Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).**

## 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

Sandwich ELISA structure (Detection antibody labeled with HRP) A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns 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:** antibody coated 96-well microplate (8 well × 12 strips) (1 plate)
- **Protein standard:** 3000 pg/bottle; lyophilized (2 bottles)

- **Detection antibody, HRP-conjugated (100×):** 120 µL/vial* (1 vial)
- **Sample Diluent PT 1-ec:** 30 mL/bottle (1 bottle)
- **Detection Diluent:** 30 mL/bottle (1 bottle)
- **Wash Buffer Concentrate (20×):** 30 mL/bottle (1 bottle)
- **Extraction Reagent:** 30 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°C. Immediately after use remaining reagents should be returned to cold storage (2 to 8°C). 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°C for 6 months or -20°C for 12 months. (See expiration date on the label.) |
| Opened kit | All reagents stored at 2 - 8°C 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.

## 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.

### 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.

### Detection Antibody, HRP-conjugated(1X)

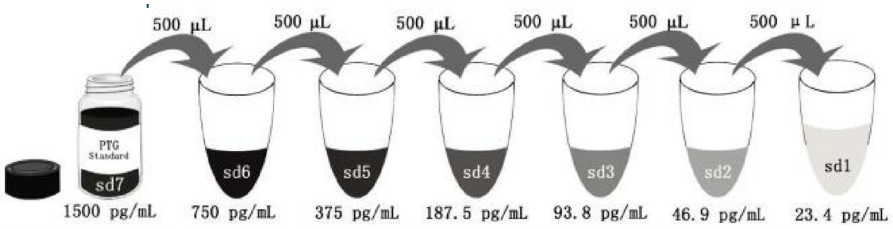
Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100 X Detection Antibody solution, HRP-conjugated for a few seconds prior to use)

### 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 and plasma; 1:2 is recommended for urine; 1:2 or 1:4 is recommended for cell lysate.

### Standard Serial Dilution

Add 2 mL Sample Diluent PT 1-ec 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-ec | **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 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.** 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.** Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

**3.** Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

**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 1X 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 of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

**6.** Repeat wash step in 8.4.

**7.** Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

**8.** Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

**9.** Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

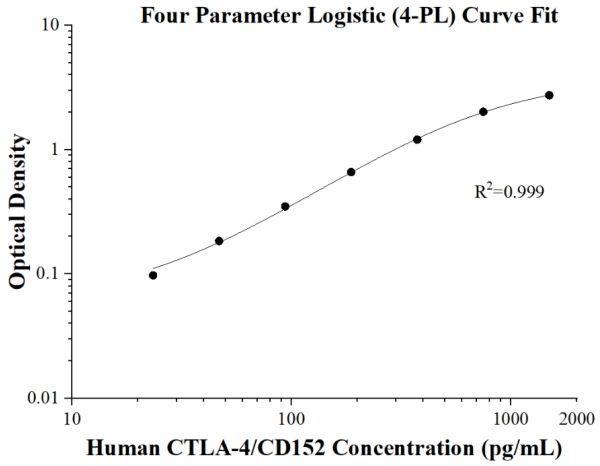
| Step | Reagent | Volume | Incuba-tion | Wash | Notes |
|---|---|---|---|---|---|
| 1 | Standard and Samples | 100 µL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 3 | Diluent Detection antibody, HRP-conjugated Solution | 100 µL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 4 | TMB Substrate | 100 µL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 4 | Stop Solution | 100 µL | 0 min | Do not wash | - |
| 5 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

## 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, use four-parameter logistic curvefit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve 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.




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.0764<br>0.0735 | 0.07495 | - |
| 23.4 | 0.1656<br>0.1792 | 0.1724 | 0.09745 |
| 46.9 | 0.2682<br>0.25 | 0.2591 | 0.18415 |
| 93.8 | 0.4499<br>0.3987 | 0.4243 | 0.34935 |
| 187.5 | 0.7785<br>0.6919 | 0.7352 | 0.66025 |
| 375 | 1.3511<br>1.2073 | 1.2792 | 1.20425 |
| 750 | 2.165<br>2.0217 | 2.09335 | 2.0184 |
| 1500 | 2.8605<br>2.7653 | 2.8129 | 2.73795 |

## SENSITIVITY

The minimum detectable dose of human CTLA-4/CD152 is 3.7 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 | 742.0 | 34.2 | 4.6 |
| 2 | 8 | 176.9 | 4.5 | 2.6 |
| 3 | 8 | 95.0 | 2.4 | 2.6 |

### Inter-assay Precision

| Sample | n | Mean (pg/mL) | SD | CV% |
|---|---|---|---|---|
| 1 | 16 | 729.6 | 46.0 | 6.3 |
| 2 | 16 | 173.5 | 6.5 | 3.7 |
| 3 | 16 | 92.1 | 5.2 | 5.6 |

## 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 serum | 1:2 | 83 | 78-87 |
| Urine | 1:2 | 99 | 92-105 |
| Cell lysate | 1:8 | 111 | 108-114 |
| | 1:16 | 113 | 110-118 |

## LINEARITY

To assess the linearity of the assay, human serum samples and urine samples were spiked with high concentrations 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 lysate samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

| | | Human serum | Urine | Cell lysate |
|---|---|---|---|---|
| 1:2 | Average% of Expected | 91 | 100 | 100 |
| | Range (%) | 90-92 | 99-101 | - |
| 1:4 | Average% of Expected | 99 | 101 | 121 |
| | Range (%) | 98-100 | 100-101 | 117-126 |
| 1:8 | Average% of Expected | 108 | 102 | 97 |
| | Range (%) | 106-110 | 102-103 | 84-110 |
| 1:16 | Average% of Expected | 110 | 109 | 82 |
| | Range (%) | 108-112 | 108-110 | 81-83 |

## CALIBRATION

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

## SAMPLE VALUES

**Human serum -** Human serum samples were evaluated for the presence of human CTLA-4/CD152 in this assay. 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

| Sample Type | Mean (pg/mL) | Detection rate | Range (pg/mL) |
|---|---|---|---|
| Human serum (n=16) | 59.2 | 50% | ND-303.0 |
| | Human CTLA-4/CD152 (pg/mL) | Total protein (mg/mL) | |
| A549 cell lysate | 3,592.9 | 2.2 | |

## 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: MCP-2 CTLA-4 MCP-3 MCP-4 GROα GROβ IL-8 PANTES

## PLATE LAYOUT

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