

EliKine™ Human CCL3 ELISA Kit

Cat #: KTE6002

Size: 48 T/96 T

	Human CCL3 ELISA Kit		
REF	Cat #: KTE6002	LOT	Lot #: Refer to product label
	Detection range: 5 pg/mL-80 pg/mL		Sensitivity: 0.5 pg/mL
	Precision: Intra-assay Precision: The CV (%) < 10%. Inter-assay Precision :The CV (%) < 12%		Recovery: The recovery ranged from 98% to 116% with an overall mean recovery of 106%.
	Specificity: EliKine™ Human CCL3 ELISA Kit has high sensitivity and excellent specificity for detection of Human CCL3. No significant cross-reactivity or interference between Human CCL3 and analogues was observed.		
	Applicable samples: Serum, Plasma, Cell culture supernatants		
	Storage: Stored at 4°C for 6 months, protected from light		

Assay Principle

Macrophage inflammatory protein-1 alpha (MIP-1 alpha), also called CCL3, LD78. The cDNA for MIP-1 alpha predicts a mature peptide of 69 amino acids with a molecular mass of 7,889 daltons. LD78 is a member of a newly identified superfamily of small inducible proteins involved in inflammatory responses, wound healing and tumorigenesis. EliKine™ Human CCL3 ELISA Kit employs a double antibody sandwich method to quantitate Human CCL3 in samples. An antibody specific for Human CCL3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Human CCL3 present is bound by the immobilized antibody. After removing any unbound substances, a HRP-conjugated antibody specific for Human CCL3 is added to the wells. After washing, remove any unbound HRP-conjugated antibody reagent, adding HRP Substrate (TMB), TMB turns blue under the catalysis of HRP, and turns yellow after adding stop solution. Measure the OD value with a microplate reader at 450 nm wavelength. The Human CCL3 concentration is proportional to the OD450 nm value.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Human CCL3 Microplate	48 wells	96 wells	4°C
Human CCL3 Standard (160 pg/mL)	0.5 mL	0.5 mL	4°C
Standard Diluent	1.5 mL	1.5 mL	4°C
HRP-conjugated Human CCL3 Detect Antibody	3 mL	6 mL	4°C
Sample Diluent	3 mL	6 mL	4°C
HRP Substrate A	3 mL	6 mL	4°C, protected from light
HRP Substrate B	3 mL	6 mL	4°C, protected from light

Stop Solution	3 mL	6 mL	4°C
Wash Buffer (20×)	20 mL (20×)	20 mL (30×)	4°C
Plate Covers	2	2	RT

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm
- Multi channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

Standard Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. Standard Diluent is used for dilution of standard.

Sample Diluent: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C. Sample Diluent is used for dilution of samples.

Human CCL3standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

HRP-conjugated Human CCL3Detect Antibody: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

HRP Substrate A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

HRP SubstrateB: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

Stop Solution: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C

1×Wash Buffer: Equilibrate to room temperature and dilute the Wash Buffer 20 times with deionized water at 48 T and 30 times with deionized water at 96 T to obtain 1×Wash Buffer before use. Mix gently to avoid foaming. Store at room temperature. Please note that 1×Wash buffer is stable for 30 days.

Standard curve setting: dilute 160 pg/mL standard with Standard Diluent to 80, 40, 20, 10 and 5 pg/mL of Human CCL3 standard just as below.

Num.	Volume of Standard	Volume of Standard Diluent (μL)	The concentration of Standard (pg/mL)
Std.1	125 μL of 160 pg/mL	125	80
Std.2	125 μL of Std.1 (80 pg/mL)	125	40
Std.3	125 μL of Std.2 (40 pg/mL)	125	20
Std.4	125 μL of Std.3 (20 pg/mL)	125	10
Std.5	125 μL of Std.4 (10 pg/mL)	125	5

Note: Always prepare a fresh set of standards per use.

Sample Preparation

1. Cell culture supernatants: Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
2. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
3. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
4. Tissue: Weigh 0.1g of the tissue, add 0.9mL of cold 0.01 M pH7.4 PBS (protease inhibitors can be added), and homogenize in an ice bath. Collect the homogenate, centrifuge at 10,000 g at 4°C for 10 min, take the supernatant and immediately measure

or aliquot and store samples at -20°C to avoid repeated freezing and thawing.

5. Cells: The adherent cells were gently washed with cold PBS, then digested with trypsin, centrifuged at 1,000 g for 5 min and collect cells; Suspended cells can be collected directly by centrifugation. The collected cells were washed 3 times with cold PBS. Add 150-200 µL of PBS to resuspend each 10⁶ cells (it is recommended to add protease inhibitors to PBS); If the content is very low, the volume of PBS can be reduced) and then ultrasonic break in ice bath for 2 min (power 20% or 200 W, ultrasound for 3 seconds, interval 7 seconds, repeated 12 times). Centrifuge the extract at 4°C and 10,000 g for 10 min. Take the supernatant and immediately measure or aliquot and store samples at -20°C to avoid repeated freezing and thawing.

6. Urine, saliva, and other liquid samples: Centrifuge 3,000 g for 10 min, take the supernatant and immediately measure or aliquot and store samples at -20°C to avoid repeated freezing and thawing.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 h, 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.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal. The strips used for testing are equilibrated to room temperature before use.

2. Set Blank well (Blank well without sample, HRP conjugated Human CCL3 Detect Antibody, all other steps are the same) and Test wells separately. Add 40 µL of Sample Diluent and 10 µL of sample (the final sample dilution is 5 times) to Test wells. Add the sample to the bottom of the enzyme-linked immunosorbent assay plate well, avoiding touching the well wall as much as possible, and gently shake and mix well. Add 50 µL of standard samples of different concentrations to the standard wells in sequence. Cover with the plate cover provided. Incubate for 30 min at 37°C.

3. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a multi channel pipette or automated microplate washer, and let it stand for 1-2 min, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.

4. Add 50 µL of HRP-conjugated Human CCL3 Detect Antibody to each well, excluding Blank well. Cover with the plate cover provided.

Incubate for 30 min at 37°C.

5. Repeat the wash as in step 3.

6. Add 50 µL of HRP Substrate A to each well first, then add 50 µL of HRP Substrate B, gently shake and mix well, and incubate for 15 min at 37°C. Protect from light.

7. Add 50 µL of Stop solution to each well. Stop Solution should be added to the plate in the same order as TMB. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

8. Determine the optical density of each well within 15 min, zero with blank well and measure the absorbance at 450 nm for each well.

Data Analysis

1. Calculate the average OD value of each standard and sample in multiple Wells.

2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

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

Typical Data

Typical standard curve ($R^2 \geq 0.99$)

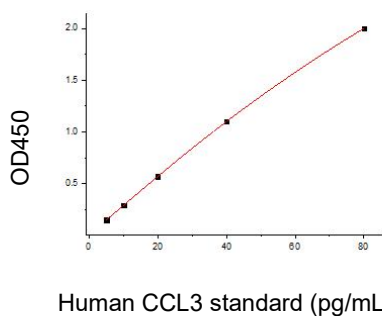


Figure1. Standard Curve of Human CCL3 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. If Standard Diluent and Sample Diluent appears to turn yellow or a small amount of precipitation, etc. Please centrifuge to remove the precipitate, which will not affect normal use.
2. Do not mix or substitute reagents with those from other lots or sources.
3. 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.
4. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
5. Stop Solution has certain Corrosive. Please take protective measures when operating.

Recommended Products

Catalog No.	Product Name
KTE6001	EliKine™ Human CCL2 ELISA Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.