Quantikine® ELISA

Human Caspase-1/ICE Immunoassay

Catalog Number DCA100

For the quantitative determination of human Caspase-1 concentrations in cell culture supernates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Caspase-1, also known as IL-1β-converting enzyme (ICE) is synthesized as an inactive precursor. Dimerization and proteolysis generate an active caspase composed of two large (~20 kDa) and two small (~10 kDa) subunits. The active enzyme contains 2 active sites. An active site cysteine is located at the carboxyl terminal of each large subunit and forms a covalent bond with substrates or inhibitors (1). Caspase-1 cleaves substrates at the carboxyl terminal of aspartate residues.

Caspase-1 proteolytically processes cellular inactive precursor IL-1β to extracellular active IL-1β, a cytokine involved in the inflammatory response (2). Mice deficient in Caspase-1 do not produce IL-1β and are resistant to endotoxic shock (3). Caspase-11 is required for activation of Caspase-1 and subsequently, Caspase-11 deficient mice fail to activate Caspase-1 or export IL-1β (4). Therefore, Caspase-1 is downstream of Caspase-11 in the IL-1β pathway. The IL-1β precursor can be cleaved by Caspase-1 after the tetrapeptide sequence Tyr^{113}-Val^{114}-Cys^{115}-Asp^{116}. Although many tetrapeptide substrates are cleaved by Caspase-1, there are additional constraints on Caspase-1 *in vivo*. Tertiary and/or quaternary substrate structure influences the susceptibility of a protein to hydrolysis by Caspase-1 (5).

In addition to proteolytically processing cytokines, Caspase-1 also appears to be involved in a caspase cascade in apoptosis and the inflammatory response. Thymocytes from Caspase-1 deficient mice are resistant to Fas-mediated apoptosis (6). Caspase-1 activation in the inflammatory response appears to involve serial activation of caspases similar to the caspase cascade that occurs in apoptosis. The involvement of Caspase-1 in initiating an inflammatory response makes Caspase-1 an attractive target for inhibitors of its activity. A Caspase-1 inhibitor has potential to suppress the inflammatory response.

The Quantikine Human Caspase-1/ICE Immunoassay is a 3 hour solid phase ELISA designed to measure Caspase-1 in cell culture supernates. It contains recombinant human Caspase-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained measuring natural human Caspase-1 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Caspase-1. These results indicate that this kit can be used to determine relative mass values for natural human Caspase-1.

PRINCIPLE OF THE ASSAY

This assay is a monoclonal/polyclonal based assay, which is specific to the p20 subunit of Caspase-1. A monoclonal antibody specific for human Caspase-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Caspase-1 present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for human Caspase-1 is added to the wells. After washing away any unbound antibody, anti-rabbit IgG-HRP conjugate is added to the wells. Following a wash to remove any unbound conjugate, a substrate solution is added and color develops in proportion to the amount of Caspase-1 bound. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Caspase-1 Microplate	890765	96 well polystyrene microplate (8 strips of 12 wells) coated with a monoclonal antibody specific for human Caspase-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human Caspase-1 Standard	890767	2 vials of recombinant human Caspase-1 with bound inhibitor in a buffered protein base with preservative; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard within 2 hours of use. Use a fresh standard for each assay.
Human Caspase-1 Antiserum	890768	11 mL of rabbit polyclonal antibody specific for human Caspase-1 in a buffered protein base with preservative.	May be stored for up to 1 month at 2-8 °C.*
Human Caspase-1 Conjugate	890766	11 mL of goat anti-rabbit IgG conjugated to horseradish peroxidase with preservative.	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human Caspase-1 Controls (optional; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage condition listed below is intended as general guideline. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

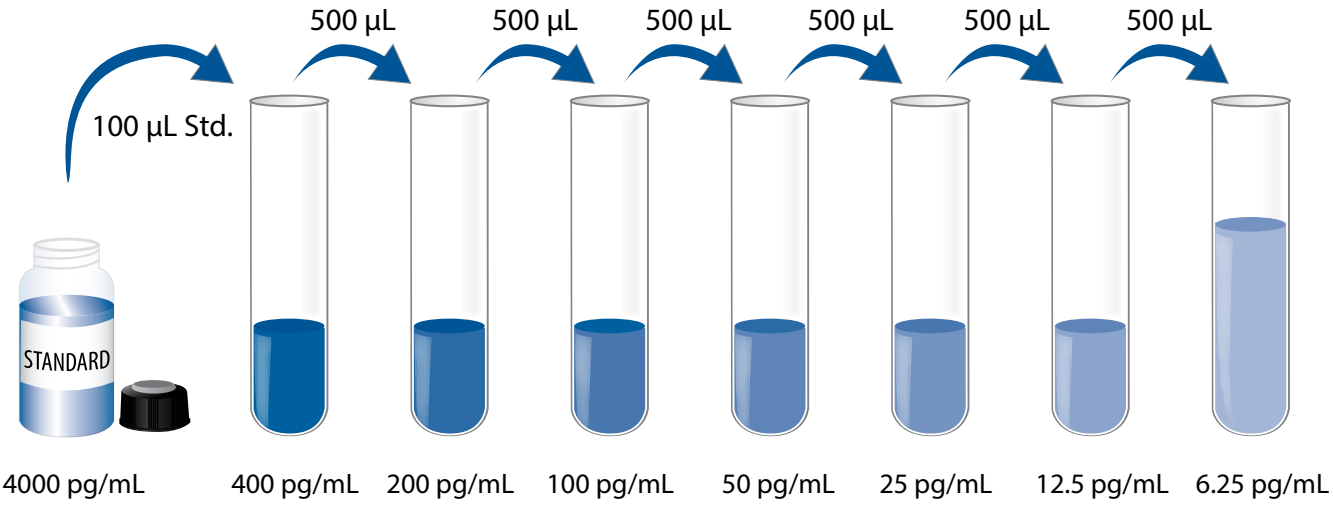
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human Caspase-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Caspase-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. **Note:** *Use within 2 hours of reconstitution.*

Pipette 900 µL Calibrator Diluent RD5-5 into the 400 pg/mL tube. Pipette 500 µL of Calibrator Diluent RD5-5 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 400 pg/mL standard serves as the high standard. Calibrator Diluent RD5-5 serves as the zero standard (0 pg/mL). **Use within 30 minutes of preparation.**



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of Standard, control, or sample per well. **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover with the adhesive strip provided. Incubate for 1.5 hours at room temperature.
5. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Caspase-1 Antiserum to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Human Caspase-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
11. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

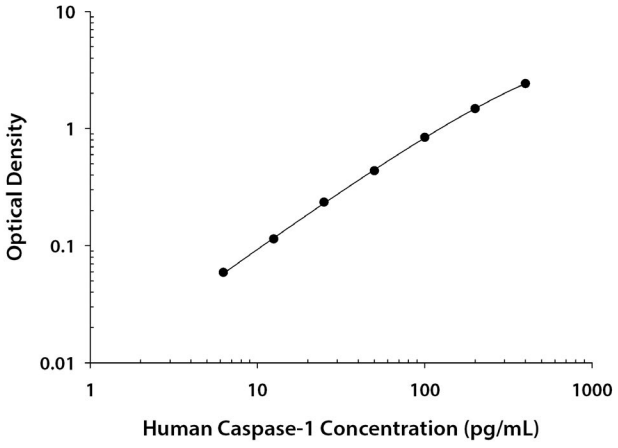
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Caspase-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.049 0.051	0.050	—
6.25	0.109 0.109	0.109	0.059
12.5	0.164 0.163	0.164	0.114
25	0.278 0.292	0.285	0.235
50	0.482 0.491	0.486	0.436
100	0.906 0.877	0.892	0.842
200	1.604 1.451	1.528	1.478
400	2.445 2.508	2.476	2.426

PRECISION

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

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	41.0	77.7	228	40.4	75.2	219
Standard deviation	2.49	4.62	11.1	3.80	6.90	18.1
CV (%)	6.1	5.9	4.9	9.4	9.2	8.3

RECOVERY

The recovery of human Caspase-1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	93	85-106%

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human Caspase-1 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Natural cell culture supernates (n=5)	Spiked cell culture supernates (n=5)
1:2	Average % of Expected	101	102
	Range (%)	100-101	92-113
1:4	Average % of Expected	101	105
	Range (%)	97-104	90-111
1:8	Average % of Expected	93	104
	Range (%)	83-103	92-116
1:16	Average % of Expected	92	105
	Range (%)	86-97	89-121

SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of human Caspase-1 ranged from 0.22-1.24 pg/mL. The mean MDD was 0.68 pg/mL..

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

SAMPLE VALUES

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated and stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human Caspase-1.

Sample Type	Day 1 (pg/mL)	Day 3 (pg/mL)	Day5 (pg/mL)
Unstimulated	42.3	131	——
Stimulated	70.7	200	87.8

SPECIFICITY

This assay recognizes natural and recombinant human Caspase-1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human Caspase-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Caspase-2
Caspase-3
Caspase-7
Caspase-8
IL-1α
IL-1β
IL-1ra
IL-1 RI
IL-1 RII
IL-10
IL-18
IL-18 (pro)

Recombinant mouse:

IL-1β

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