

Quantikine[®]

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 (IL-1 β -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-1 β to extracellular active IL-1 β , a cytokine involved in the inflammatory response (2). Mice deficient in caspase-1 do not produce IL-1 β 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-1 β (4). Thus, caspase-1 is downstream of caspase-11 in the IL-1 β pathway. The IL-1 β precursor can be cleaved by caspase-1 after the tetrapeptide sequence Tyr¹¹³-Val¹¹⁴-Cys¹¹⁵-Asp¹¹⁶. 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 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 the Quantikine 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 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 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.
- 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.
- This assay is designed to eliminate interference by enzymes and proteins present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Caspase-1 Microplate (Part 890765) - 96 well polystyrene microplate (8 strips of 12 wells) coated with a monoclonal antibody against Caspase-1.

Caspase-1 Standard (Part 890767) - 3 vials of recombinant human Caspase-1 with bound inhibitor in a buffered protein base with preservative; lyophilized.

Caspase-1 Antiserum (Part 890768) - 11 mL of rabbit polyclonal antibody against Caspase-1 in a buffered protein base with preservative.

Caspase-1 Conjugate (Part 890766) - 11 mL of goat anti-rabbit IgG conjugated to horseradish peroxidase with preservative.

Assay Diluent RD1W (Part 895117) - 11 mL of a buffered protein base with preservatives.

Calibrator Diluent RD5-5 (Part 895485) - 21 mL of a buffered protein base with preservatives.

Wash Buffer Concentrate (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative.

Color Reagent A (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

Color Reagent B (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

Stop Solution (Part 895032) - 6 mL of 2 N sulfuric acid.

Plate Covers - 4 adhesive strips.

STORAGE

| | | |
|---|---|--|
| Unopened Kit | Store at 2 - 8° C. Do not use past kit expiration date. | |
| Opened/ Reconstituted Reagents | Diluted Wash Buffer | May be stored for up to 1 month at 2 - 8° C.* |
| | Stop Solution | |
| | Assay Diluent RD1W | |
| | Calibrator Diluent RD5-5 | |
| | Antiserum | |
| | Unmixed Color Reagent A | |
| | Unmixed Color Reagent B | |
| | Conjugate | |
| | Standard | Discard within 2 hours. Use a fresh standard for each assay. |
| | Microplate Wells | Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.* |

*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.
- Human Caspase-1 Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

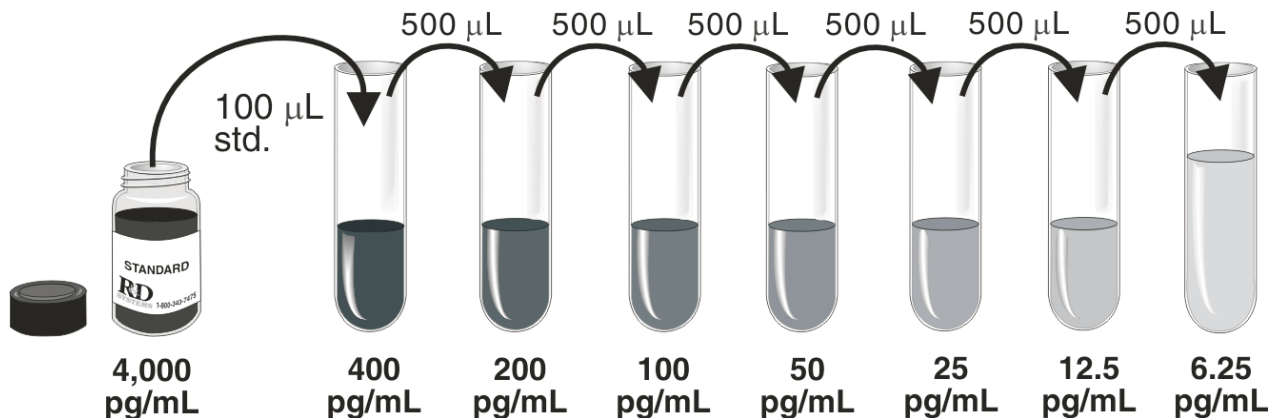
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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.

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. **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 samples, controls, and standards 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 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.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as instructed.



2. Add 50 μL Assay Diluent RD1W to each well.



3. Add 100 μL Standard, control or sample to each well **within 15 minutes**. Incubate 1.5 hours at RT.



4. Wash 3 times.



5. Add 100 μL Antiserum to each well.
Incubate 30 minutes at RT.



6. Wash 3 times.



7. Add 100 μL of Conjugate to each well.
Incubate 30 minutes at RT.



8. Wash 3 times.



9. Add 200 μL of Substrate Solution to each well.
Incubate 20 minutes at RT. **Protect from light.**



10. Add 50 μL Stop Solution to each well.
Read at 450 nm within 30 minutes.
 λ correction 540 or 570 nm

CALCULATION OF RESULTS

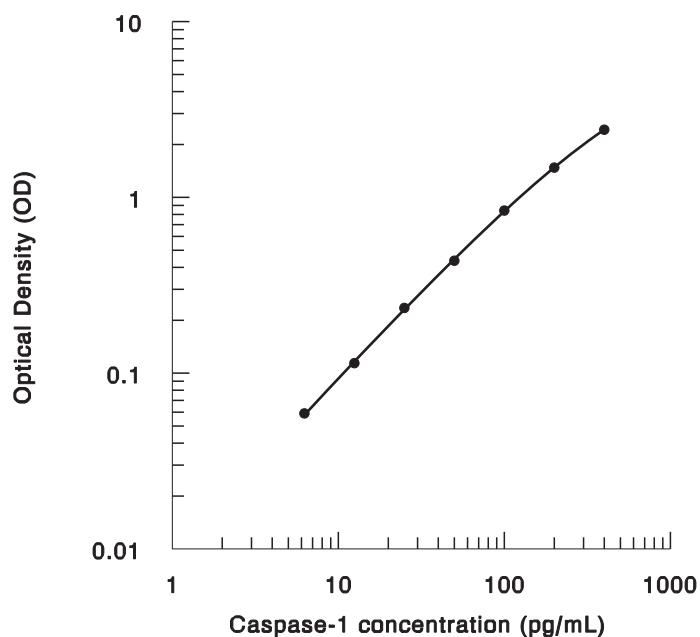
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 Caspase-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

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

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.
- 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.
- 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.

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.

| Sample | Intra-assay Precision | | | Inter-assay Precision | | |
|--------------------|-----------------------|------|-------|-----------------------|------|------|
| | 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 | 11.11 | 3.8 | 6.9 | 18.1 |
| CV (%) | 6.1 | 5.9 | 4.9 | 9.4 | 9.2 | 8.3 |

RECOVERY

The recovery of Caspase-1 spiked to three different levels in four cell culture media samples throughout the range of the assay was evaluated. The mean recovery was 93% and ranged from 85 - 106%.

LINEARITY

To assess the linearity of the assay, cell culture supernate samples containing and/or spiked with high concentrations of Caspase-1 were diluted with Calibrator Diluent RD5-5 to produce samples with values within the dynamic range of the assay.

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

SENSITIVITY

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

The minimum detectable dose 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

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

| Condition | Day 1 (pg/mL) | Day 3 (pg/mL) | Day 5 (pg/mL) |
|--------------|---------------|---------------|---------------|
| Unstimulated | 42.3 | 131 | — |
| Stimulated | 70.7 | 200 | 87.8 |

SPECIFICITY

This assay recognizes both natural and recombinant human Caspase-1. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-5 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:

| | |
|---------------|----------------------|
| ANG | IL-1 sRII |
| AR | IL-2 |
| Caspase-2 | IL-2 sR α |
| Caspase-3 | IL-3 |
| Caspase-7 | IL-3 sR α |
| Caspase-8 | IL-4 |
| CNTF | IL-4 sR |
| β -ECGF | IL-5 |
| EGF | IL-5 sR α |
| Epo | IL-5 sR β |
| FGF acidic | IL-6 |
| FGF basic | IL-6 sR |
| FGF-4 | IL-7 |
| FGF-5 | IL-8 |
| FGF-6 | IL-9 |
| G-CSF | IL-10 |
| GM-CSF | IL-11 |
| sgp130 | IL-12 |
| GRO α | IL-13 |
| GRO β | IL-18 |
| GRO γ | IL-18 pro |
| HB-EGF | KGF |
| HGF | LAP (TGF- β 1) |
| IFN- γ | LIF |
| IGF-I | MCP-1 |
| IGF-II | M-CSF |
| IL-1 α | MIP-1 α |
| IL-1 β | MIP-1 β |
| IL-1ra | β -NGF |
| IL-1 sRI | OSM |

Recombinant mouse:

GM-CSF
IL-1 α
IL-1 β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1 α
MIP-1 β
SCF
TNF- α

Recombinant amphibian:

TGF- β 5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF- β 1
porcine TGF- β 1
porcine TGF- β 2

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