Human Caspase 3 Instant ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of human Caspase 3

Catalog Number BMS2012INST (128 tests)

Pub. No. MAN0016469 **Rev.** A.0 (30)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Human Caspase 3 Instant ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human Caspase 3 in cell extracts.

Summary

Caspases are a family of aspartate-specific cysteine proteases that act in a step-wise signaling manner like kinases.

Caspases are present in all cells, recruitment of these proteases to oligomerized receptors leads to activation accompanied by autoproteolytic cleavage. Active caspases can proteolyze additional caspases generating a caspase cascade that cleaves proteins critical for cell survival. The final outcome of this signaling pathway is a form of controlled cell death termed apoptosis. The subgroup of caspases involved in apoptosis is called initiators or effectors. Caspase 3 cleaves substrate at the carboxyl terminus of aspartate residues. Active Caspase 3 has two active sites and consists of two identical large (~20 kDa) and two identical small (~10 kDa) subunits that are derived from two precursor Caspase 3 polypeptides. Caspase 3 is proteolytically activated by other caspases.

Both subunits contribute to substrate binding and catalysis. The active site cysteine that covalently binds the substrate is located near the C–terminus of the large subunit. Active Caspase 3 has two-fold symmetry, two active site pockets each residing on an opposite side. Caspase 3, together with Caspases 8 and 9, is situated at pivotal junctions in apoptotic pathways. Caspase 3 appears to amplify Caspase 8 and 9 initiation signals into complete commitment to apoptotic disassembly.

For literature update refer to our website.

Principles of the test

An anti-human Caspase 3 monoclonal coating antibody is adsorbed onto microwells. Human Caspase 3 present in the sample or standard binds to antibodies adsorbed to the microwells; a polyclonal anti-human Caspase 3 detection antibody (rabbit) binds to human Caspase 3 captured by the first antibody. Anti-rabbit-IgG-HRP binds to the polyclonal anti-human Caspase 3 detection antibody.

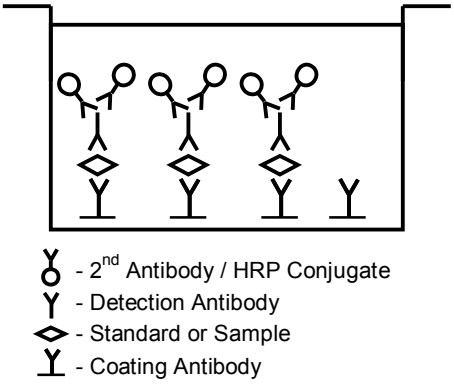

Fig. 1 First incubation.

Following incubation unbound anti-human Caspase 3 detection antibody and anti-rabbit-IgG-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

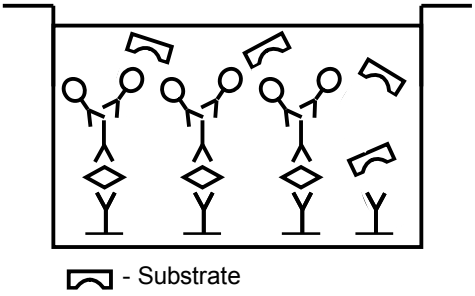

Fig. 2 Second incubation.

A colored product is formed in proportion to the amount of soluble human Caspase 3 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human Caspase 3 standard dilutions and human Caspase 3 sample concentration determined.

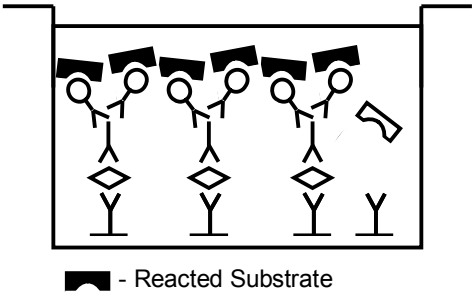

Fig. 3 Stop reaction.

Reagents provided

1 aluminum pouch with a Microwell Plate (12 strips of 8 wells each) coated with Monoclonal Antibody (murine) to human Caspase 3, Detection Antibody (rabbit anti-human Caspase 3 polyclonal antibody), Anti-rabbit-IgG- HRP, and Sample Diluent, lyophilized

2 aluminum pouches with a human Caspase 3 Standard curve (colored)

1 bottle (25 mL) Wash Buffer Concentrate 20x (phosphate-buffered saline with 1% Tween™ 20)

1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)

1 vial (12 mL) Sample Diluent (Use when an external predilution of the samples is needed)

1 vial (15 mL) Stop Solution (1M Phosphoric acid)

1 vial (15 mL) Lysis Buffer 10x

2 adhesive Plate Covers

Storage instructions

Store ELISA plate and standard curves or whole kit at –20°C. The plate and the standard curves can also be removed, stored at –20°C, and remaining kit reagents can be stored between 2°C to 8°C. Expiry of the kit and reagents is stated on labels. The expiry of the kit components can be guaranteed only if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Sample preparation

Cell extracts are suitable for use in the assay. Prepare cell extracts after induction of apoptosis. Numerous extraction protocols can be used. The following protocol is provided as an example of a suitable extraction procedure, but should not be construed as necessarily being the method of choice. Users may wish to experiment with extraction procedures that work best in their hands.

Cell Lysate protocol:

1. For suspension cells, pellet by centrifugation, remove supernatant and proceed to step 3. For attached cells, remove supernatant from cells.
2. Wash cells once with PBS; harvest cells by scraping and gentle centrifugation.
3. Aspirate PBS leaving an intact cell pellet (at this point the cell pellet can be frozen at –80°C and lysed at a later date). We recommend for every 5×10^6 cells, resuspend the pellet in 1 mL of Lysis Buffer.
4. Incubate 60 minutes at room temperature with gentle shaking.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 1000 x *g* for 15 minutes.
6. Aliquot cleared lysate to clean microfuge tubes. These samples are now ready for analysis according to the instructions provided in Test Protocol. Lysates can be frozen at –80°C and assayed at a later time. The sample should be divided into small aliquots to avoid multiple freeze/thaw cycles.

For stability and suitability of samples refer to “Performance characteristics“ on page 4.

Note: Samples found to contain greater than 100 ng/mL Caspase 3 (i.e., outside the range of the standard curve) must be diluted with Sample Diluent (provided), so that the Caspase 3 concentration falls within the range spanned by the standard curve, and assayed again.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents and samples

1. Buffer concentrate should be brought to room temperature and diluted before starting the test procedure.
2. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

Wash buffer (1x)

- Pour entire contents (25 mL) of the Wash Buffer Concentrate (20x) into a clean 500 mL graduated cylinder. Bring to final volume to 500 mL with glass-distilled or deionized water. Mix gently to avoid foaming.
- Transfer to a clean wash bottle and store at 2°C to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

Lysis buffer (1x)

Pour the entire contents (15mL) of the Lysis Buffer Concentrate (10x) into a clean 150 mL graduated cylinder. Bring to final volume of 150 mL with distilled or deionized water and mix gently. Store at room temperature.

Test protocol

Note:

- Use plate immediately after removal from –20°C!
- Do not wait until pellets have completely dissolved before applying samples; the binding reaction in the standard strips starts immediately after addition of water!
- Do not try to dissolve pellets by pipetting up and down in the wells; some parts of the pellet could stick to the tip creating high variation of results
- Perform the washing step with at least 400 µL of washing buffer as stated in the manual or fill the wells completely; otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results
- Allow the washing buffer to sit in the wells for a few seconds before aspiration
- Remove covers of the standard strips carefully in order that all the lyophilised pellets remain in the wells

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

1. Determine the number of microwell Strips required to test the desired number of samples plus microwell Strips for blanks and standards (colored). Each sample, standard, blank, and optional control sample should be assayed in duplicate. Remove extra microwell Strips from holder and store in foil bag with the desiccant provided at –20°C sealed tightly. Place microwell strips containing the standard curve in position A1/A2 to H1/H2 (see Table 1 on page 3).
2. Add distilled water to all standard and blank wells as indicated on the label of the standard strips (A1/A2 to H1/H2).
3. Add 140 µL of distilled water to the sample wells.

   Table 1 Example of the arrangement of blanks, standards, and samples in the microwell strips.

	1	2	3	4
A	Standard 1 10.00 ng/mL	Standard 1 10.00 ng/mL	Sample 1	Sample 1
B	Standard 2 5.00 ng/mL	Standard 2 5.00 ng/mL	Sample 2	Sample 2
C	Standard 3 2.50 ng/mL	Standard 3 2.50 ng/mL	Sample 3	Sample 3
D	Standard 4 1.25 ng/mL	Standard 4 1.25 ng/mL	Sample 4	Sample 4
E	Standard 5 0.63 ng/mL	Standard 5 0.63 ng/mL	Sample 5	Sample 5
F	Standard 6 0.31 ng/mL	Standard 6 0.31 ng/mL	Sample 6	Sample 6
G	Standard 7 0.16 ng/mL	Standard 7 0.16 ng/mL	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

4. Add 10 µL of each Sample, in duplicate, to the designated wells and mix the contents.
5. Cover with a Plate Cover and incubate at room temperature (18°C to 25°C) for 3 hours on a microplate shaker.
6. Remove Plate Cover and empty wells. Wash the microwell strips 6 times with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
7. After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.
8. Pipette 100 µL of TMB Substrate Solution to all wells, including the blank wells.
9. Incubate the microwell strips at room temperature (18°C to 25°C) for about 10 minutes. Avoid direct exposure to intense light.

   The color development on the plate should be monitored and the substrate reaction stopped (see step 10) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

   It is recommended to add the stop solution when the highest standard has developed a dark blue color. The color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.
10. Stop the enzyme reaction by quickly pipetting 100 µL of Stop Solution into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2°C to 8°C in the dark.
11. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human Caspase 3 standards.

Calculation of results

1. Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20% of the mean.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human Caspase 3 concentration on the abscissa. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating human Caspase 3 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human Caspase 3 concentration.
4. Samples have been diluted 1:10, thus the concentration read from the standard curve must be multiplied by the dilution factor (× 10). According to the suggested Lysis Protocol the determined concentration can also be referred to the cell number (1 mL = 5×10^6 cells).

   Note: There is a common dilution factor for samples due to the conjugate, which must then be included in the calculation. The samples contribute 100 µL to the final volume per well. These 100 µL are composed of 90 µL of sample diluent plus 10 µL of the sample. This is a 1:10 dilution.

   The remaining 50 µL to give 150 µL are due to the addition of 50 µL conjugate to all wells.

   90 µL sample diluent and 50 µL conjugate results in 140 µL reconstitution volume, addition of 10 µL sample (90 µL + 10 µL = 1:10 dilution).
5. Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human Caspase 3 levels (possible Hook Effect). Such samples require further external predilution according to expected human Caspase 3 values with Sample Diluent in order to precisely quantitate the actual human Caspase 3 level.

6. It is suggested that each testing facility establishes a control sample of known human Caspase 3 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
7. A representative standard curve is shown in Figure 4 on page 4.

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

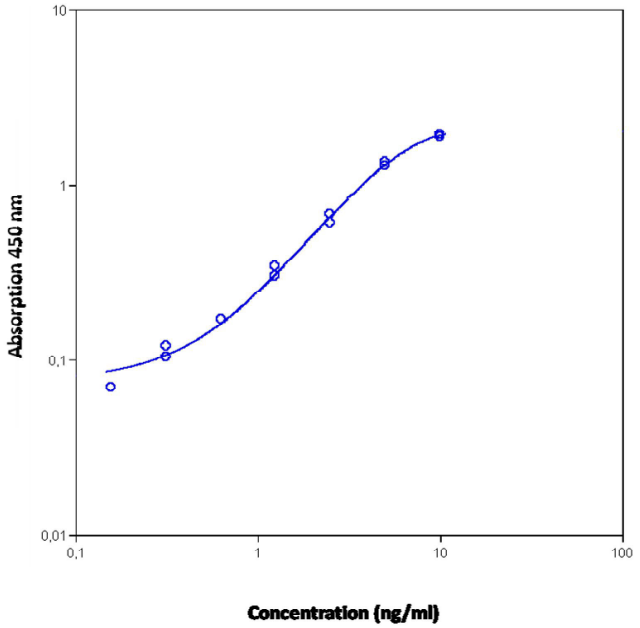

Fig. 4 Representative standard curve Human Caspase 3 Instant ELISA Kit. Human Caspase 3 was diluted in serial 2-fold steps in Sample Diluent, each symbol represents the mean of 3 parallel titrations.

Table 2 Typical data using the Human Caspase 3 Instant ELISA Kit.

Measuring wavelength: 450 nm

Reference wavelength: 620 nm

Standard	Human Caspase 3 concentration (ng/mL)	O.D. (450 nm)	O.D. mean	C.V. (%)
1	10.00	1.922 1.866	1.894	1.5
2	5.00	1.343 1.278	1.311	2.5
3	2.50	0.609 0.674	0.642	5.1
4	1.25	0.300 0.341	0.321	6.4
5	0.63	0.171 0.171	0.171	0.0
6	0.31	0.104 0.120	0.112	7.1
7	0.16	0.070 0.069	0.070	0.7
Blank	0.00	0.045 0.044	0.045	1.1

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Because exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks, or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle, and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analyzed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

Performance characteristics

Sensitivity

The limit of detection of human Caspase 3 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.12 ng/mL (mean of 6 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 4 lysate samples containing different concentrations of human Caspase 3. Two standard curves were run on each plate. Data below show the mean human Caspase 3 concentration and the coefficient of variation for each sample (see Table 3 on page 4). The calculated overall intra-assay coefficient of variation was 7.7%.

Table 3 The mean human Caspase 3 concentration and the coefficient of variation for each sample.

Positive sample	Experiment	Human Caspase 3 Concentration (ng/mL)	Coefficient of variation (%)
1	1	31.3	5.0
	2	31.4	9.0
	3	27.5	5.0
2	1	17.2	9.0
	2	15.7	7.0
	3	15.9	9.0
3	1	53.8	9.0
	2	56.5	3.0
	3	59.0	10.0
4	1	97.2	9.0
	2	105.3	10.0
	3	97.4	8.0

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments by 3 technicians. Each assay was carried out with 6 replicates of 4 lysate samples containing different concentrations of human Caspase 3. Two standard curves were run on each plate. Data below (see Table 4 on page 5) show the mean human Caspase 3 concentration and the coefficient of variation

calculated on 18 determinations of each sample. The calculated overall coefficient of variation was 5.4%.

Table 4 The mean human Caspase 3 concentration and the coefficient of variation calculated on 18 determinations of each sample.

Sample	Human Caspase 3 concentration (ng/mL)	Coefficient of variation (%)
1	30.1	7.4
2	16.2	5.0
3	56.5	4.6
4	100.0	4.6

Dilution parallelism

Four lysate samples with different levels of human Caspase 3 were analyzed at serial 2-fold dilutions with 4 replicates each. The recovery ranged between 88.3% and 118.1% with an overall recovery of 105.0%.

Sample	Dilution	Human Caspase 3 concentration (ng/mL)		Recovery of exp. val. (%)
		Expected value	Observed value	
1	1:10	–	28.4	–
	1:20	14.2	15.8	111.4
	1:40	7.9	7.5	94.5
	1:80	3.7	3.3	88.3
2	1:10	–	75.1	–
	1:20	37.5	38.8	103.4
	1:40	19.4	21.4	110.1
	1:80	10.7	12.4	116.4
3	1:10	–	64.3	–
	1:20	32.1	34.1	106.0
	1:40	17.0	19.4	114.2
	1:80	9.7	11.5	118.1
4	1:10	–	31.5	–
	1:20	15.8	16.5	104.6
	1:40	8.2	8.0	96.6
	1:80	4.0	3.9	96.7

Sample stability

Freeze-thaw stability

Aliquots of lysate samples (unspiked or spiked) were stored at –20°C and thawed 5 times, and the human Caspase 3 levels determined. There was no significant loss of human Caspase 3 immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of lysate samples (spiked or unspiked) were stored at –20°C, 2°C to 8°C, room temperature, and at 37°C, and the human Caspase 3 level determined after 72 hours. There was no significant loss of human Caspase 3 immunoreactivity detected during storage at –20°C and 4°C. Storage at room temperature showed 20%, and storage at 37°C showed 30% loss of Caspase 3 immunoreactivity.

Reagent preparation summary

Wash buffer (1x)

Add Wash Buffer Concentrate 20x (25 mL) to 475 mL distilled water.

Lysis buffer (1x)

Add Lysis Buffer Concentrate 10x (15 mL) to 135 mL distilled water.

Test protocol summary

Note: Samples have been diluted 1:10, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 10).

1. Place standard strips in position A1/A2 to H1/H2.
2. Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
3. Add 140 µL distilled water to sample wells.
4. Add 10 µL Sample to designated wells.
5. Cover microwell strips and incubate 3 hours at room temperature (18°C to 25°C) on a microplate shaker.
6. Empty and wash microwell strips 6 times with 400 µL Wash Buffer.
7. Add 100 µL of TMB Substrate Solution to all wells including blank wells.
8. Incubate the microwell strips for about 10 minutes at room temperature (18°C to 25°C).
9. Add 100 µL Stop Solution to all wells including blank wells.
10. Blank microwell reader and measure color intensity at 450 nm.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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