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ProductInformation

Survivin ELISA, Human

Product Number **CS0240**
Storage Temperature 2-8 °C

Technical Bulletin

Product Description

Survivin ELISA is a complete kit for the quantitative determination of Survivin levels in serum, plasma, urine, and cell lysates. A monoclonal antibody specific for human Survivin has been coated onto the wells of the multiwell plate provided. Survivin standard dilutions, control specimens, and unknown samples are pipetted into these wells. During the first incubation, the Survivin antigen binds to the immobilized (capture) antibody. After incubation, excess reagents are washed away and a rabbit polyclonal antibody to Survivin is added. This antibody binds to the immobilized Survivin protein captured during the first incubation. After removal of excess reagents goat anti-rabbit IgG conjugated to horseradish peroxidase is added. This binds to the polyclonal Survivin antibody and completes the sandwich. After a third incubation and washing to remove excess anti-rabbit IgG-HRP, substrate solution is added, which is acted upon by the bound enzyme to produce a color. The enzyme reaction is stopped and the color generated is read at 450 nm. The measured optical density is directly proportional to the concentration of Survivin in either standards or samples.

The Survivin ELISA detects specifically human Survivin protein. There is less than 0.1% cross reactivity with human MEK-1, pJNK, p300, Granzyme B, Caspase-3, or Caspase-9. The cross reactivity with rat and mouse Survivin in this ELISA is currently under investigation and you may contact Sigma Technical Service department for more information.

Survivin is a 16.5 kDa protein and the smallest inhibitor of apoptosis so far identified. It is involved in the inhibition of apoptosis and cell division. Survivin expression has been reported at high levels in embryonic tissues, but at low or non-detectable levels in normal tissue. [1] Survivin regulates the G2/M phase of the cell cycle by associating with the mitotic spindle microtubules and directly inhibiting Caspase-3 and Caspase-7. Survivin is selectively expressed in the most common human cancers and is associated with clinical tumor progression. [2] It has been proposed as a tumor marker for breast cancer and Survivin expression has been correlated to clinical outcome in melanoma patients. [3,4] Down-regulation or loss of Survivin is thought to inhibit the growth of tumor cells. Further, it has been indicated that Survivin epitopes may serve as important targets for anticancer immunotherapy approaches, and that Survivin is a rational target for apoptosis-based cancer therapy. It has also been proposed that Survivin may be used as a universal tumor antigen for immunotherapy. [5]

Reagents

- **Survivin Standard, Human, 2 vials, 500 pg each, Product No. S-8943-** lyophilized recombinant human Survivin
- **Monoclonal Anti-Survivin-coated 96 well plate, 1 plate, Product No. S 8818 -** A plate using break-apart strips coated with monoclonal antibody specific for human Survivin.
- **Anti-Survivin Antibody**, **11 mL**, **Product No. S 9193 -** a yellow colored solution of polyclonal antibody from rabbit.
- **Anti-Rabbit IgG-Horseradish Peroxidase (HRP**), **11 mL, Product No. I 5908 –** a blue solution. Ready to use.
- **Assay Buffer #20, 120 mL, Product No. A 4103 –** Use for standard reconstitution and standard and samples dilutions (before use add inhibitors).
- **Wash Buffer #2 Concentrate, 10X, 100 mL, Product No. W 3014 –** Tris buffered saline containing detergents
- **TMB Substrate, 12 mL, Product No. T 8449-** A solution of TMB and hydrogen peroxide. Avoid exposure to light. Avoid exposure to metals.
- **Stop Solution, 11 mL, Product No. S 9068 –** 1 N HCl, keep tightly capped.
- **Cell Lysis Buffer #2, 100 mL, Product No. C 0365**
- **Plate Covers, 3 EA, Product No. P 1496**

Reagents and Equipment required but not provided

- Deionized or distilled water.
- Precision pipettes for volumes between 100 mL and 1,000 mL.
- Repeater pipette for dispensing 100 mL.
- Disposable beakers for diluting buffer concentrates.
- Graduated cylinders.
- A multiwell plate shaker.
- Adsorbent paper for blotting.
- A multiwell plate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
- Graph paper for plotting the standard curve.
- Phenylmethylsulfonylfluoride (PMSF) (Product No. P 7626)
- Protease inhibitor cocktail (PIC) (Product No. P 2714).
- Survivin Cell Dilution Buffer. The buffer contains: 0.137 M sodium chloride, 2.7 mM potassium chloride, 8.1 mM sodium phosphate dibasic, 1.5 mM potassium phosphate dibasic, 1% bovine serum albumin, pH 7.3.

Precautions and Disclaimer

The kit is for R&D use only, not for drug, household or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

- Samples of choice –cell culture media, serum, heparinized plasma, urine, cell lysates and other biological fluids.
- Samples should be separated from the cells and frozen if not analyzed shortly after collection.
- Sample matrix has a dramatic impact on Survivin recovery. To ensure accurate quantitation, the standard curves and blanks must be generated in the same diluent as the samples.
- Diluent of choice: Assay Buffer 20 + Inhibitors
- Based on the recovery data, the recommended dilutions of samples in the Assay Buffer 20 plus Inhibitors are as follows:
    - Cell lysates - 1:80
    - Serum and urine - 1:2
    - Heparin plasma - 1:4
- Cell culture samples could be read in the assay, provided the standards, samples and controls are diluted in the cell culture media or appropriate buffer.
- Keep samples on ice until ready to apply to plate.
- Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible avoid use of hemolyzed or lipemic sera.
- Make samples dilutions in polypropylene tubes.
- Avoid use of sodium azide, thymol or thimerosal as sample preservatives.

Cell Lysis Buffer #2 (Product No. C 0365)

- Cell Lysis Buffer contains:
  1 mM EDTA
  6 M Urea
  0.5% Triton X-100
  0.005% Tween 20 in phosphate buffered saline
- Immediately before use add 100 µM PMSF (Product No. P 7626)
- Immediately before use add 0.05% (Product No. P 2714).

Protocol for Cell Lysis

This is an example of lysis protocol. The researchers may choose any lysis protocol described in literature or used in the laboratory.

1. Collect cells in PBS by centrifugation (non-adherent) or scraping from culture flasks (adherent).
2. Wash twice with cold PBS.
3. Remove and discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at –70 ºC and lysed at a later date).
4. Lyse the cell pellet in Cell Lysis Buffer for 30 minutes on ice with vortexing at 10-minute intervals. The volume of Cell Lysis Buffer depends on the cell number in cell pellet.
5. Transfer extracts to microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4 ºC.
6. Aliquot the clear lysate to clean microcentrifuge tubes.
7. Prior to assaying the cell lysates in the ELISA they must be diluted 1:6 in Cell Dilution Buffer (see the formulation in Reagents Required But Not Provided).
8. The lysates in Cell Dilution Buffer are further diluted 1:80 in Assay Buffer #20 with inhibitors.

Reagent Preparation

Survivin Standard

1. Equilibrate the lyophilized 500 pg/vial standard to room temperature for not more then 10 minutes.
2. Reconstitute with 0.5 mL of Assay Buffer #20 with inhibitors or with cell culture media (for cell culture samples).
3. Mix gently and wait 5 minutes to ensure complete reconstitution.
4. Label as **1,000 pg/mL Survivin Standard**.
5. Prepare serial standard dilutions as follows:

Tube #	Assay Buffer 20 + Inhibitors	Standard from tube #:	Final Standard Concentration pg/mL
1	Reconstitute according to label instructions		1000 pg/mL
2	0.25 mL	0.25 mL (1)	500
3	0.25 mL	0.25 mL (2)	250
4	0.25 mL	0.25 mL (3)	125
5	0.25 mL	0.25 mL (4)	62.5
6	0.25 mL	0.25 mL (5)	31.25

6. Use diluted standards within 20 minutes

Assay Buffer #20 with inhibitors

1. This buffer is used for standard reconstitution and for dilutions of standards and samples (with the exception of cell culture samples, which are diluted in cell media or buffers).
2. Take out the amount of Assay Buffer needed for the assay run.
3. Immediately before assay add 1 mM PMSF (Product No. P 7626).
4. Immediately before assay add 250 µL of reconstituted PIC (Product No. P 2714).
5. For each ELISA assay make a fresh Assay Buffer #20 with inhibitors.

Wash Buffer #2

1. Dilute 100 mL of Wash Buffer concentrate into 900 mL deionized water
2. Store at room temperature for up to 3 months or for the entire shelf life of the kit.

Storage/Stability

All components of this kit are stable at 2 - 8 ºC. Any unused reconstituted standard should be discarded or frozen at 70 ºC. Standard can be frozen and thawed one time only without loss of immunoreactivity.
Refer to the Certificate of Analysis for kit shelf life. To obtain C of A go to www sigma-aldrich.com

Procedure

Precautions

- 20-30 minutes before use equilibrate kit and all reagents to room temperature (15-30 ºC).
- Use only the coated 96 multiwell capture plate provided with the kit.
- Multiwell plate: equilibrate to room temperature in unopened foil bag. Remove desired number of strips, reseal the bag and refrigerate at 2 - 8°C to maintain plate integrity.
- When not in use all kit components should be refrigerated.
- Assay all standards, controls and samples in duplicate.
- If particulate matter is present, centrifuge or filter prior to analysis.
- A standard curve must be run with each assay
- Maintain a consistent order of components and reagents addition from well to well. This ensures equal incubation times for all wells.
- Run in-house controls with every assay. If control values fall outside pre-established ranges, the accuracy of the assay may be suspect.
- All reagents are lot-specific. Do not mix reagents from different kit lots.
- Do not use reagents after the kit expiration date.
- Standards and samples can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent and use fresh pipette tips for each sample, standard or reagent.
- Read absorbances within 2 hours of assay completion.

Washing directions

- The purpose of washing is to remove unbound proteins and other non-specific parts of lysate.
- Incomplete washing will adversely affect the assay and render false results.
- Use only Wash Buffer #2 provided in kit.
- Washing may be performed using automated washer, manifold pipette or squirt bottle.
- Wash cycle five times, blotting as dry as possible after the 5th wash.
- When washing manually, fill wells with Wash Buffer, aspirate thoroughly and tap dry on absorbent tissue.
- It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing and drying procedure to avoid strips coming free of the frame.

Assay Procedure

- Determine the number of wells for the assay run, including 2 chromogen blank wells, 2 assay buffer blank wells, 12 standard dilutions wells and 2 wells for each sample to be assayed. Remove the appropriate number of multiwell strips and return the unused strips to the pouch. Reseal pouch and refrigerate.

SUMMARY OF SURVIVIN ELISA

1. Add 100 µL of Standards, Samples (diluted as in Sample Preparation) and Assay Buffer Blank
Incubate 1 hr at RT on horizontal shaker at 500 rpm

Aspirate and wash 5X

2. Add 100 µL Anti-Survivin Antibody to all wells (except chromogen blank)
Incubate 1 hr at RT on horizontal shaker at 500 rpm

Aspirate and wash 5X

3. Add 100 µL of Anti-Rabbit IgG-HRP to all wells (except chromogen blank)
Incubate 30 minutes at RT on horizontal shaker at 500 rpm

Aspirate and wash 5X

4. Add 100 µL of TMB substrate to all wells
Incubate 30 minutes at RT on horizontal shaker at 500 rpm

5. Add 100 µL of Stop Solution to all wells and read at 450 nm

Total Time 3 hours

1st Incubation
a. Add 100 µL of Assay Buffer, standard and samples to the appropriate wells (except the two chromogen blanks).
b. Cover the plate and incubate for 1 hour with shaking at room temperature.
c. Wash wells 5 times following washing instructions.
d. After the final wash, blot dry on a lint free paper towel to remove any remaining wash buffer.

2nd Incubation
a. Add 100 µL of Anti-Survivin antibody to all wells (except 2 chromogen blanks).
b. Cover the plate and incubate for 1 hour with shaking at room temperature.
c. Wash wells 5 times following washing instructions.
d. After the final wash, blot dry on a lint free paper towel to remove any remaining wash buffer.

3rd Incubation
a. Add 100 µL of Anti-rabbit IgG-HRP conjugate to all wells (except chromogen blanks).
b. Cover the plate and incubate for 30 minutes with shaking at room temperature.
e. Wash wells 5 times following washing instructions.
c. After the final wash, blot dry on a lint free paper towel to remove any remaining wash buffer.

Substrate incubation
a. Add 100 µL TMB substrate to all wells
d. Incubate for 30 minutes with shaking at room temperature.

Stop reaction
a. Add 100 µL of Stop Solution to each well. This stops the reaction
b. Tap gently to mix. *The solution will turn yellow*

Absorbance reading
a. Any commercially available multiwell plate reader capable of reading at OD 450 nm may be used.
b. Blank the plate reader against the Chromogen Blank wells (contain Chromogen and Stop Solution).
c. Read absorbance at OD 450 nm

Results

The results may be calculated using any immunoassay software package. The four-parameter algorithm provides the best curve fit. If the software program is not readily available, the concentrations of Survivin may be calculated as follows:

1. Calculate the Average Net OD (nm) for each standard dilution and samples as follows:
   Average Net OD (nm)=
   Average Bound OD (nm) –
   Average Chromogen Blank OD (nm)
2. On graph paper plot the Average Net OD (nm) of standard dilutions against the concentration (pg/mL) of Survivin for the standards.
3. Draw the best curve through these points to construct the standard curve.
4. The Survivin concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by dilution factor of each sample.
6. Samples producing signals higher than the 1,000 pg/mL standard should be further diluted in Assay Buffer and re-assayed.

Product Profile

Typical Results

The standard curve below is for illustration only and should not be used to calculate results in your assay. Run standard curve in each assay.

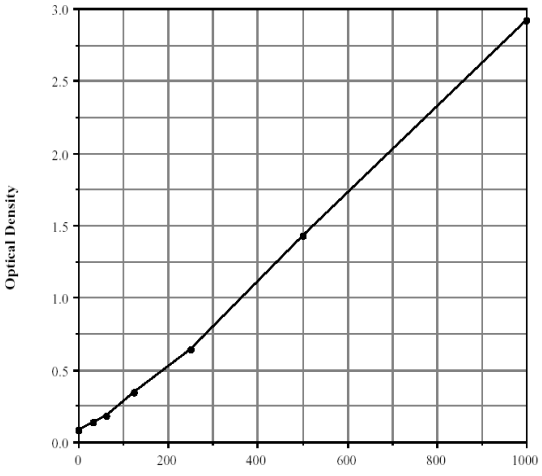
Tube #	Survivin Standard pg/mL	Net OD 450 nm
1	1,000	2.921
2	500	1.426
3	250	0.644
4	125	0.340
5	62.5	0.183
6	31.25	0.134
	Sample #1 693	2.004
	Sample #2 89.1	0.265

Limitations

- Do not extrapolate the standard curve beyond the 1000 pg/mL standard point.
- The dose response is non-linear in this region and accuracy will be compromised.
- Other buffers and matrices have not been investigated
- Although Survivin degradation in the Cell Extraction Buffer described in this protocol has not been seen to date, the possibility of this occurrence cannot be excluded.

Typical Standard Curve

- The curve shown below is for the illustration purpose and must never be used to calculate Survivin concentrations.
- Each researcher must run his own standard curve



Typical Survivin Standard Curve

Performance Characteristics

Sensitivity

Sensitivity was calculated by determining the average optical density for sixteen (16) wells run with 0 pg/mL Standard, and comparing to the average optical density for sixteen (16) wells run with Standard #6.

- The detection limit was determined as the concentration of human Survivin measured at two standard deviations from the 0 pg/mL standard along the standard curve.

Mean OD for Chromogen blank = 0.081 ± 0.003 (3.3%)
Mean OD for Standard #6 =0.133 ± 0.005 (4.0%)

Delta Optical Density
(31.25 - 0 pg/mL) = 0.133- 0.081= 0.052
2 SD's of 0 pg/mL Standard 2 x 0.003 = 0.006
Sensitivity 0.006 x 31.25 pg/mL = 3.6 pg/mL

Linearity

A sample containing 464.1 pg/mL human Survivin was serially diluted 4 times 1:2 in the Assay Buffer #20 and measured in the assay. The data was plotted graphically as actual Survivin concentration versus measured Survivin concentration. The line obtained had a slope of 0.966 with a correlation coefficient of 0.999.

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of human Survivin and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium, and high concentrations of Survivin in multiple assays (n=11). The precision numbers listed below represent the percent coefficient of variation for the concentrations of human Survivin determined in these assays as calculated by a 4 parameter logistic curve-fitting program.

	Survivin pg/mL	**Intra-assay %CV**	**Inter-assay %CV**
Low	117	2.4%	
Medium	217	1.3%	
High	693	1.8%	
Low	142		15.9%
Medium	278		17.5%
High	700		5.9%

Cross reactivity

The human Survivin human ELISA is specific for human Survivin. There is less than 0.1% cross-reactivity with human MEK-1, pJNK, p300, Granzyme B, Caspase-3, or Caspase-9. Cross-reactivity for rat and mouse Survivin in this ELISA are currently under evaluation. Contact us for suitability of applications to these sample types.

Recovery

Human Survivin concentrations were measured in tissue culture media, cell lysates, serum, plasma and urine. Undiluted samples of these matrices were spiked with human Survivin then diluted with the appropriate diluent and assayed in the kit.

The following results were obtained:

Sample	Recovery	Recommended Dilution
Tissue Culture Media	107.1	none
Cell Lysis Buffer 2/ Cell lysates	99.3	1:80
Serum	108	1:2
Heparin Plasma	106	1:4
Urine	88.1	1:2

References

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3. Nasu, S., et al., Survivin mRNA expression in patients with breast cancer. Anticancer Res., **22,** 1839-1843 (2002).
4. Gradilone, A., et al., Survivin, bcl-2, bax, and bcl-X gene expression in sentinel lymph nodes from melanoma patients. J. Clin. Oncol., **21**, 306-312 (2003).
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