Standard is now desiccated

Survivin (human), ELISA kit

Catalog No. ADI-900-111

96 Well Kit

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Description

The Survivin (human), ELISA kit is a complete kit for the quantitative determination of Survivin in serum, plasma, urine, and cell lysates. Please read the complete kit insert before performing this assay. The kit uses a monoclonal antibody to Survivin immobilized on a microtiter plate to bind the Survivin in the standards or samples. A recombinant Survivin Standard is provided in the kit. After a short incubation the excess sample or standard is washed out and a rabbit polyclonal antibody to Survivin is added. This antibody binds to the Survivin captured on the plate. After a short incubation the excess antibody is washed out and goat anti-rabbit IgG conjugated to Horseradish peroxidase is added, which binds to the polyclonal Survivin antibody. Excess conjugate is washed out and substrate is added. After a short incubation, 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. For further explanation of the principles and practices of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

Introduction

Survivin is a 16.5 kDa protein and the smallest inhibitor of apoptosis (IAP) 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 [3]. Survivin regulates the G2/M phase of the cell cycle by associating with the mitotic spindle microtubules and directly inhibits Caspase-3 and Caspase-7.

Survivin is selectively expressed in the most common human cancers and is associated with clinical tumor progression[4]. It has been proposed as a tumor marker for breast cancer, and Survivin expression has been correlated to clinical outcome in melanoma patients [5,6]. 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[7].

Precautions

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1. Stop Solution 2 is a 1N hydrochloric acid solution. This solution is caustic; care should be taken in use.
2. The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine.
3. We test this kit's performance with a variety of buffers, however it is possible that high levels of interfering substances may cause variation in assay results.
4. The human Survivin Standard provided, Catalog No. 80-1035, should be handled with care because of the known and unknown effects of Survivin.

Materials Supplied

1. **Survivin Microtiter Plate, One Plate of 96 Wells, Catalog No. 80-1026**
   A plate using break-apart strips coated with a mouse monoclonal antibody specific to Survivin.
2. **Total Survivin Antibody, 10 mL, Catalog No. 80-1028**
   A yellow solution of rabbit polyclonal antibody to human Survivin.
3. **Assay Buffer 20, 120 mL, Catalog No. 80-1029**
4. **Total Survivin Conjugate, 10 mL, Catalog No. 80-1324**
   A blue solution of goat anti-rabbit IgG conjugated to Horseradish peroxidase.
5. **Wash Buffer Concentrate, 100 mL, Catalog No. 80-1287**
   Tris buffered saline containing detergents.
6. **human Survivin Standard, 1 bottle, Catalog No. 80-1710**
   One bottle containing two vials in desiccant, 500 pg each lyophilized recombinant human Survivin.
7. **TMB Substrate, 10 mL, Catalog No. 80-0350**
   A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. **Protect from prolonged exposure to light.**
8. **Stop Solution 2, 10 mL, Catalog No. 80-0377**
   A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**
9. **Cell Lysis Buffer 2, 100 mL, Catalog No. 80-1037**
   1 mM EDTA, 6 M Urea, 0.5% Triton X-100, 0.005% Tween 20 in Phosphate Buffer Saline.
10. **Cell Dilution Buffer Concentrate, 1 each, Catalog No. 80-1036**
    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, pH7.3.
11. **human Total Survivin Assay Layout Sheet, 1 each, Catalog No. 30-0181**
12. **Plate Sealer, 3 each, Catalog No. 30-0012**

Storage

All components of this kit are stable at 4°C until the kit's expiration date. The Standard **must** be stored in the original bottle.

Materials Needed but Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 100 µL and 1,000 µL.
3. Repeater pipet for dispensing 100 µL.
4. Disposable beakers for diluting buffer concentrates.
5. Graduated cylinders.
6. A microplate shaker.
7. Adsorbent paper for blotting.
8. Microplate reader capable of reading at 450 nm, preferably with correction between 570 nm and 590 nm.
9. Graph paper for plotting the standard curve.
10. Phenylmethyl Sulfonyl Fluoride (PMSF), Sigma #P7626 or equivalent.
11. Protease inhibitor cocktail (PIC), Sigma #P8340 or equivalent.

Sample Handling

The Survivin (human), ELISA is compatible with human Survivin samples in a wide range of cell lysates, serum, plasma, urine, and buffers. Samples diluted sufficiently into Assay Buffer 20 plus Inhibitors (see Reagent Preparation, page 5, #2) can be read directly from a standard curve. Please refer to the Sample Recovery recommendations on page 11 for details of suggested dilutions.

Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the Tissue Culture Media instead of Assay Buffer 20 plus Inhibitors. There will be a small change in binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of human Survivin in the appropriate matrix.

It is recommended that all cells be lysed with the provided Cell Lysis Buffer 2 modified by the addition of PMSF and PIC (see Reagent Preparation, page 5, #4). This buffer is 1 mM EDTA, 6 M Urea, 0.5% Triton X-100, and 0.005% Tween 20 in Phosphate Buffer Saline. Samples lysed in the provided Cell Lysis Buffer 2 plus Inhibitors **must** be diluted 1:6 in Cell Dilution Buffer modified by the addition of PMSF and PIC (see Reagent Preparation, page 5, #5) prior to further dilution in Assay Buffer 20 plus Inhibitors.

If the end user chooses to use their own Cell Lysis Buffer, it is up to the end user to determine the appropriate dilution of samples and assay validation.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. The Survivin Standard should not be left at room temperature for extended periods of time. A maximum of 15 minutes at room temperature is recommended.
4. Standards must be made up in polypropylene tubes.
5. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
6. Pipet standards and samples to the bottom of the wells.
7. Add the reagents to the side of the well to avoid contamination.
8. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4 °C in the sealed bag provided. The wells should be used in the frame provided.
9. **Prior to addition of antibody, conjugate and substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**
10. **It is important that the matrix for the standards and samples be as similar as possible. Dilute human Survivin samples with Assay Buffer 20 plus Inhibitors then run with a standard curve diluted in the same buffer. Tissue Culture samples should be read against a standard curve diluted in the same complete but non-conditioned media. See Reagent Preparation, step 2.**

Reagent Preparation

1. **Wash Buffer**
   Prepare the Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. **Assay Buffer 20 plus Inhibitors**
   Immediately prior to use in the assay, PIC and PMSF must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.

   This modified Assay Buffer 20 must be used for standard reconstitution and all sample and standard dilutions (unless Tissue Culture Media is being run as a sample) to ensure optimal integrity of Survivin. Fresh Assay Buffer 20 plus Inhibitors <u>must</u> be made for each assay.

3. **Survivin Standards**
   Allow the 500 pg/vial human Survivin standard to warm for no more than 10 minutes. Label five 12x75 mm polypropylene tubes #2 through #6. Pipet 500 µL of standard diluent (Assay Buffer 20 plus Inhibitors or Tissue Culture Media) into Survivin Standard vial. Pipet 250 µL of standard diluent into tubes #2 though #6. Add 250 µL of the 1,000 pg/mL Standard to tube #2. Vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #6.

   The concentration of Survivin in the reconstituted human Survivin Standard vial and in tubes #2 through #6 will be 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL respectively. See Survivin Assay Layout Sheet for dilution details. Diluted standards should be used within 20 minutes of preparation.

4. **Cell Lysis Buffer 2 plus Inhibitors**
   Allow to come to room temperature. Immediately prior to cell lysis, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.

   Fresh Cell Lysis Buffer 2 plus Inhibitors must be made each time the cells are lysed.

5. **Cell Dilution Buffer plus Inhibitors**
   Allow to come to room temperature. Prepare Cell Dilution Buffer for use by diluting 10 mL of the supplied concentrate with 90 mL of deionized water. Immediately prior to use, PMSF and PIC must be added to the buffer. If using Sigma Protease Inhibitor Cocktail #P8340, add 0.5 µL/mL or equivalent concentration according to alternate vendor's specification sheet. Add PMSF, such as Sigma #P7626, to a final concentration of 1 mM.

   Fresh Cell Dilution Buffer plus Inhibitors must be made each time samples are prepared for use.

Assay Procedure

Bring all reagents (with the exception of the Survivin Standard) to room temperature for at least 30 minutes prior to opening.

All standards, controls and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 100 µL of standard diluent (Assay Buffer 20 plus Inhibitors or Tissue Culture Media) into the S0 (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Tap the plate gently to mix the contents.
6. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
7. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
8. Pipet 100 µL of yellow Antibody into each well, except the Blank.
9. Seal the plate and incubate at room temperature on a plate shaker for 1 hour at ~500 rpm.
10. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 100 µL of blue Conjugate to each well, except the Blank.
12. Seal the plate and incubate at room temperature on a plate shaker for 30 minutes at ~500 rpm.
13. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 4 more times for a total of **5 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
14. Pipet 100 µL of Substrate Solution into each well.
15. Incubate for 30 minutes at room temperature on a plate shaker at ~500 rpm.
16. Pipet 100 µL Stop Solution 2 to each well. This stops the reaction and the plate should be read immediately.
17. Blank the plate reader against the Blank wells, read the optical density at 450 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all the readings.

Calculation of Results

Several options are available for the calculation of the concentration of Survivin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of Survivin can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average Blank OD

2. Using linear graph paper, plot the Average Net OD for each standard versus Survivin concentration in each standard. Approximate a straight line through the points. The concentration of Survivin in the unknowns can be determined by interpolation.

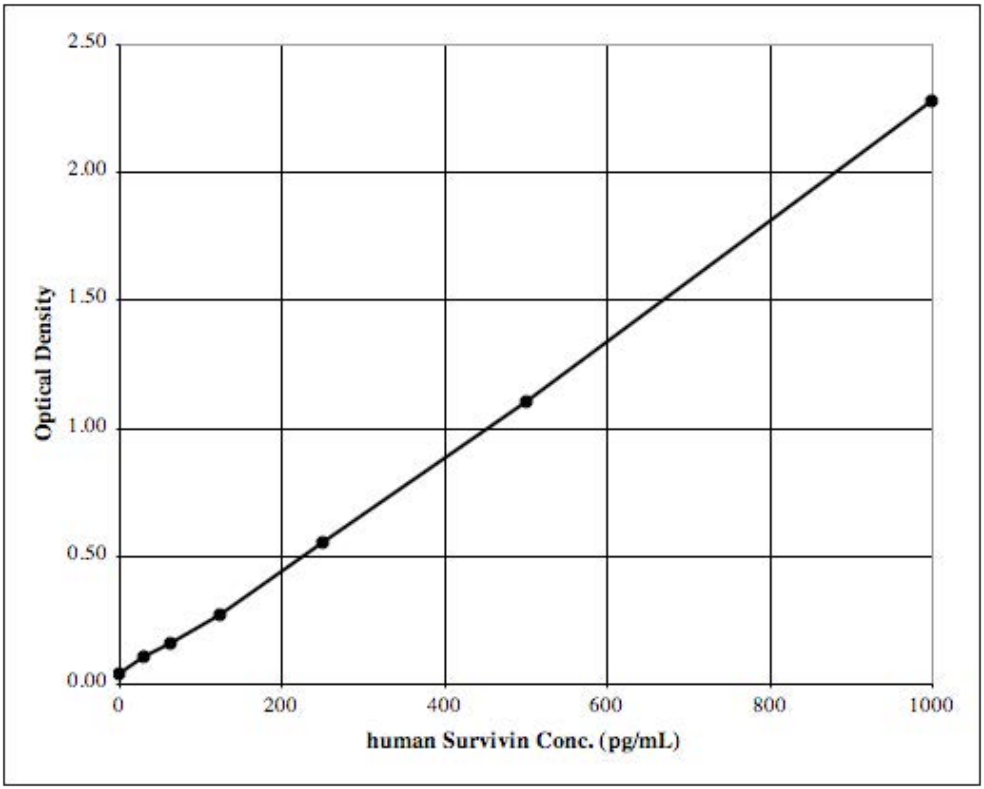
Typical Results

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Sample	Average OD	Net OD	**Survivin (pg/mL)**
Blank	0.050		
S0	0.88	0.038	**0**
S1	2.324	2.274	**1,000**
S2	1.153	1.103	**500**
S3	0.598	0.548	**250**
S4	0.315	0.265	**125**
S5	0.207	0.157	**62.5**
S6	0.151	0.101	**31.25**
Unknown #1	1.444	1.394	**626**
Unknown #2	0.208	0.158	**65.6**

Typical Standard Curve

A typical standard curve is shown below. This curve **must not** be used to calculate Survivin concentrations; each user must run a standard curve for each assay.



Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[8].

Sensitivity

Sensitivity was calculated by determining the average optical density bound 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 (2) standard deviations from the 0 pg/mL Standard along the standard curve.

Mean OD for S0 =	0.065 ± 0.004 (5.8%)
Mean OD for Standard #6 =	0.124 ± 0.005 (4.3%)
Delta Optical Density (31.25 - 0 pg/mL) = 0.124 - 0.065=	0.059
2 SD's of 0 pg/mL Standard = 2 x 0.004 =	0.008

Sensitivity = $\frac{0.008}{0.059}$ x 31.25 pg/mL = **4.0 pg/mL**

Linearity

A sample containing 826.6 pg/mL human Survivin was serially diluted 5 times 1:2 in the Assay Buffer 20 supplied in the kit 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 1.025 with a correlation coefficient of 0.997.

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=20) 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=9).

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	58	7.2	
Medium	355	2.8	
High	569	7.4	
Low	66		12.0
Medium	372		13.5
High	628		11.7

Cross Reactivities.

The human Total Survivin ELISA kit 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.

We are currently evaluating cross-reactivities of rat and mouse Survivin in this kit. Contact us for suitability of applications to these sample types.

Sample Recoveries

Please refer to pages 4 and 5 for Sample Handling recommendations and Standard preparation.

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
Jurkat Cell Lysate 4 million cells/mL	106	≥1:80
Serum	108	≥1:2
Heparin Plasma	106	≥1:4
Urine	88.1	≥1:2

* See Sample Handling instructions on page 4 for details.

****WARNING: If the end user chooses to not use the provided Cell Lysis Buffer 2, it is up the end user to determine the appropriate dilution of samples and assay validation for their chosen cell lysis buffer.**

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
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