

DRG[®] Survivin (human) ELISA (EIA-4137)

Revised 30 Jan. 2006

**DESCRIPTION**

DRG[®]'s human Total Survivin Enzyme Immunometric Assay (EIA) 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 Chard¹ or Tijssen².

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 tissue³. 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 progression⁴. 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 immunotherapy⁷.

PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1. Stop Solution 2 is a 1 normal (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, should be handled with care because of the known and unknown effects of Survivin.

REAGENTS SUPPLIED**1. Survivin Microtiter Plate, One Plate of 96 Wells**

A plate using break-apart strips coated with a mouse monoclonal antibody specific to Survivin.

2. human Total Survivin Antibody, 11 mL

A yellow solution of rabbit polyclonal antibody to human Survivin 1 & 2.

DRG[®] Survivin (human) ELISA (EIA-4137)**Revised 30 Jan. 2006****3. Assay Buffer 20, 120 mL****4. human Total Survivin Conjugate, 11 mL**

A blue solution of goat anti-rabbit IgG conjugated to Horseradish peroxidase.

5. Wash Buffer 2 Concentrate, 100 mL

Tris buffered saline containing detergents.

6. human Survivin Standard, 2 each

Two vials containing 500 pg each lyophilized recombinant human Survivin.

7. TMB Substrate, 12 mL

A solution of 3,3',5,5' tetramethylbenzidine (TMB) and hydrogen peroxide. **Protect from prolonged exposure to light.**

8. Stop Solution 2, 11 mL

A 1N solution of hydrochloric acid in water. Keep tightly capped. Caution: **Caustic.**

9. Cell Lysis Buffer 2, 100 mL

1 mM EDTA, 6 M Urea, 0.5% Triton X-100, 0.005% Tween 20 in Phosphate Buffer Saline.

10. human Total Survivin Assay Layout Sheet, 1 each**11. Plate Sealer, 3 each****MATERIALS REQUIRED BUT NOT PROVIDED****Materials Needed but Not Supplied**

1. Deionized or distilled water.
2. Precision pipets for volumes between 100 mL and 1,000 mL.
3. Repeater pipet for dispensing 100 mL.
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)
11. Protease inhibitor cocktail (PIC)
12. Cell Dilution Buffer. This buffer (Catalog No. 80-1036) may be purchased from DRG[®] or prepared. The formulation is: 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.

STORAGE CONDITIONS

All components of this kit are stable at 4°C until the kit's expiration date.

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**SPECIMEN COLLECTION AND PREPARATION**

DRG[®] EIA is compatible with human Survivin samples in a wide range of cell lysis matrices, serum, plasma, urine, and buffers. Samples diluted sufficiently into Assay Buffer 20 can be read directly from a standard curve. Please refer to the Sample Recovery recommendations 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. 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. This buffer is 1mM EDTA, 6 M Urea, 0.5% Triton X-100, and 0.005% Tween 20 in Phosphate Buffer Saline (100 mM PMSF and 0.05% PIC **must** be added by the end user prior to use). Samples lysed in the provided Cell Lysis Buffer 2 **must** be diluted 1:6 in Cell Dilution Buffer prior to further dilution in Assay Buffer 20. Cell Dilution Buffer is available from DRG[®] or the formulation is shown in item #12 under “Materials Needed but Not Supplied.” **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. Maximum of 15 minutes at Room temperature is recommended.
4. Standards can be made up in either glass or plastic 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 foil bag. 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 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 2**

Prepare the Wash Buffer 2 by diluting 100 mL of the supplied concentrate with 900 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

2. Survivin Standards

Allow the 500 pg/vial human Survivin standard solution to warm for no more than 10 minutes. Label five 12x75 mm glass tubes #2 through #6. Pipet 500 µL of standard diluent (Assay Buffer 20 or Tissue Culture Media) into Survivin Standard

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vial. Pipet 250 mL of standard diluent into tubes #2 through #6. Add 250 mL of the 500 pg/vial Standard to tube #2. Vortex thoroughly. Add 250 mL 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.

3. Cell Lysis Buffer 2

Allow to come to Room temperature. Measure out the amount of Cell Lysis Buffer 2 needed for assay. Add 100 mM Phenylmethyl Sulfonyl Fluoride (PMSF), and 0.05% Protease Inhibitor Cocktail (PIC). Ensure Cell Lysis Buffer 2 is completely in solution prior to 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 foil pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 100 mL of standard diluent (Assay Buffer 20 or Tissue Culture Media) into the S0 (0 pg/mL standard) wells.
3. Pipet 100 mL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 mL 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 mL 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 mL 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 mL 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 mL 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 mL 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 mL of Substrate Solution into each well.
15. Incubate for 30 minutes at room temperature on a plate shaker at ~500 rpm.
16. Pipet 100 mL 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.

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CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of Protein G 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 Protein G 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..

$$\text{Average Net OD} = \text{Average OD} - \text{Average Blank OD}$$

2. Using linear graph paper, plot the Average Net OD for each standard versus Protein G concentration in each standard. Approximate a straight line through the points. The concentration of Protein G 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	0.090		
S0	0.167	0.077	0
S1	3.011	2.921	1,000
S2	1.516	1.426	500
S3	0.734	0.644	250
S4	0.430	0.340	125
S5	0.273	0.183	62.5
S6	0.224	0.134	31.25
Unknown 1	2.094	2.004	693.3
Unknown 2	0.355	0.265	89.1



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ASSAY CHARACTERISTICS

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols⁸.

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 =	0.081 ± 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 = $\frac{0.006}{0.052} \times 31.25$ 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 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 0.966 with a correlation coefficient of 0.9993.

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. Interassay 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	117.1	2.4	
Medium	216.5	1.3	
High	693.0	1.8	
Low	142.0		15.9
Medium	278.0		17.5
High	700.1		5.9

Cross Reactivities.

The Total Survivin EIA kit is specific for human Survivin. There is less than 0.1% crossreactivity 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.

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Sample Recoveries

Please refer to Sample Handling recommendations and Standard preparation. Human Survivin concentrations were measured in Tissue Culture Media, Cell Lysates, Serum, Plasma (heparin), Urine, and kit Assay Buffer 20. 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 *
TCM	107.1	
Cell Lysates	9.3	≥1:80
Serum	108	≥1:2
Plasma (heparin)	106	≥1:4
Urine	88.1	≥1:2

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

* See Sample Handling instructions for details.

REFERENCES

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6. A. Gradilone, et al., J. Clin. Oncol., (2003) 21(2):306-12.
7. J.D. Gordan, et al., Cytotherapy., (2002) 4(4):317-27.
8. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, 1989, NCCLS, Villanova, PA, 19085.

LIMITED WARRANTY

DRG[®] warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose. DRG[®] must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if DRG[®] is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.