DuoSet® IC

Human Total Survivin

Catalog Number DYC647-2
DYC647-5
DYC647E

For the development of sandwich ELISAs to measure Survivin in cell lysates.

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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PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of sandwich ELISAs to measure Survivin in cell lysates. An immobilized capture antibody binds Survivin present in samples or standards. After washing away unbound material, a biotinylated detection antibody specific for Survivin is used to detect the protein, utilizing a standard Streptavidin-HRP format.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

			Vials Provided	
Description	**Part #**	**Storage Conditions**	**Cat. # DYC647-2**	**Cat. # DYC647-5**
Total Survivin Capture Antibody	840471	2-8° C	1	2
Total Survivin Detection Antibody	840472	2-8° C	1	2
Total Survivin Standard	840473	2-8° C	3	5
Streptavidin-HRP	890803	2-8° C	1	1

DYC647-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*
DYC647-5 contains sufficient materials to run ELISAs on at least five 96 well plates.*

This kit is also available in an Economy Pack (R&D Systems, Catalog # DYC647E). Economy Packs contain sufficient materials to run ELISAs on 15 microplates.* Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

*Provided the following conditions are met:

- The reagents are prepared as described in this package insert.
- The assay is run as described in the General ELISA Protocol on page 6.
- The recommended microplates, buffers, diluents, substrates, and solutions are used.

OTHER MATERIALS REQUIRED

- Aprotinin (Sigma # A6279)
- Leupeptin (Tocris # 1167)
- Pepstatin (Tocris # 1190)
- Phenylmethylsulfonylfluoride (PMSF) (Sigma # P7626)
- Sodium Azide (NaN_3) (Sigma # S2002)
- Triton™ X-100 (Sigma # T9284)
- Urea
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates [Costar EIA Plates (Catalog # 2592 or R&D Systems Catalog # DY990) are suggested]
- Plate sealers (R&D Systems, Catalog # DY992)
- Squirt bottle, manifold dispenser, or automated microplate washer

SOLUTIONS REQUIRED

PBS - 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 μm filtered.

Wash Buffer - 0.05% Tween® 20 in PBS, pH 7.2-7.4 (R&D Systems, Catalog # WA126).

Block Buffer - 1% BSA,* 0.05% NaN_3, in PBS, pH 7.2-7.4.

IC Diluent #1 - 1% BSA* in PBS, pH 7.2-7.4, 0.2 μm filtered.

IC Diluent #2 - 1% BSA*, 1 M urea in PBS, pH 7.2-7.4, 0.2 μm filtered.

Note: *Approximately 50 mL of this diluent is required to run the assay on one plate.*

IC Diluent #5 - 1 mM EDTA, 0.5% Triton X-100, 6 M urea in PBS, pH 7.2-7.4.

Lysis Buffer #2 - 1 mM EDTA, 0.5% Triton X-100, 6 M urea, 10 μg/mL Leupeptin, 10 μg/mL Pepstatin, 100 μM PMSF, 3 μg/mL Aprotinin in PBS, pH 7.2-7.4.

Substrate Solution - 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) (R&D Systems, Catalog # DY999).

Stop Solution - 2 N H_2SO_4 (R&D Systems, Catalog # DY994).

*The use of R&D Systems Reagent Diluent Concentrate 2 (Catalog # DY995) or Millipore Bovine Serum Albumin, Fraction V, Protease free (Catalog # 82-045) is recommended. All buffers containing BSA must be stored at 2-8 °C.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Total Survivin Capture Antibody (Part 840471) - Each vial contains 72 μg/mL of rabbit anti-human Survivin antibody when reconstituted with 200 μL of PBS. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

Total Survivin Detection Antibody (Part 840472) - Each vial contains 7.2 μg/mL of biotinylated rabbit anti-human Survivin antibody when reconstituted with 1.0 mL of IC Diluent #1. After reconstitution, store at 2-8° C for up to 30 days or aliquot and store at ≤ -20° C in a manual defrost freezer or at ≤ -70° C for up to 3 months.*

Total Survivin Standard (Part 840473) - Each vial contains 200 ng/mL of recombinant human Survivin when reconstituted with 500 μL of IC Diluent #5. **Use a fresh standard for each assay. Use within one hour of reconstitution.** Immediately before use, an initial 6-fold dilution should be made in IC Diluent #1. Further dilutions should be made in IC Diluent #2 immediately before use. A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 pg/mL is recommended.

Streptavidin-HRP (Part 890803) - 1.0 mL of Streptavidin conjugated to horseradish-peroxidase. Store at 2-8° C. **DO NOT FREEZE.**

*Provided this is within the expiration date of the kit.

PREPARATION OF SAMPLES

Cell Lysates - Rinse cells two times with PBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x 10^7 cells/mL in Lysis Buffer #2 and allow samples to sit on ice for 15 minutes. Assay immediately or store at ≤ -70° C. Before use, centrifuge samples at 2000 x g for 5 minutes and transfer the supernate into a clean test tube. Sample protein concentration may be quantified using total protein assay. For assaying, dilute lysates 6-fold with IC Diluent #1 and make further serial dilutions in IC Diluent #2.

Note: *The final concentration of urea in all samples and standards should be 1 M prior to addition to the plate.*

PRECAUTION

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

TECHNICAL HINTS AND LIMITATIONS

- This DuoSet IC ELISA should not be used beyond the expiration date on the kit label.
- Individual results may vary due to differences in technique, plasticware and water sources.
- It is important that the diluents selected for reconstitution and for dilution of the standard reflect the environment of the samples being measured. The diluents suggested in this protocol should be suitable for most cell lysates.
- The type of enzyme and substrate and the concentrations of capture/detection antibodies used can be varied to create an immunoassay with a different sensitivity and dynamic range. A basic understanding of immunoassay development is required for the successful use of these reagents in immunoassays.
- A thorough and consistent wash technique is essential for proper assay performance. Wash Buffer should be dispensed forcefully and removed completely from the wells by aspiration or decanting. Remove any remaining Wash Buffer by inverting the plate and blotting it against clean paper towels.
- Use a fresh reagent reservoir and pipette tips for each step.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay. Buffers containing protein should be made under aseptic conditions and stored at 2-8° C or be prepared fresh daily.

GENERAL ELISA PROTOCOL

A plate layout is provided to record standards and samples assayed.

Plate Preparation

1. Dilute the Capture Antibody to the working concentration of 0.4 μg/mL in PBS without carrier protein. Immediately coat a 96 well microplate with 100 μL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash with Wash Buffer, repeating the process two times for a total of 3 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 for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of Block Buffer to each well. Incubate at room temperature for 1-2 hours.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 μL of sample or standards in IC Diluent #2 per well. Use IC Diluent #2 as the zero standard. Cover with a plate sealer and incubate 2 hours at room temperature.

   Note: *A seven point standard curve using 2-fold serial dilutions and a high standard of 4000 pg/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Dilute the Detection Antibody to a working concentration of 200 ng/mL in IC Diluent #1 immediately before use. Add 100 μL of the diluted Detection Antibody to each well. Cover with a new plate sealer and incubate 2 hours at room temperature.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Immediately before use, dilute the Streptavidin-HRP to the working concentration specified on the vial label using IC Diluent #1. Add 100 μL of the diluted Streptavidin-HRP to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2 of the Plate Preparation.
7. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, 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.

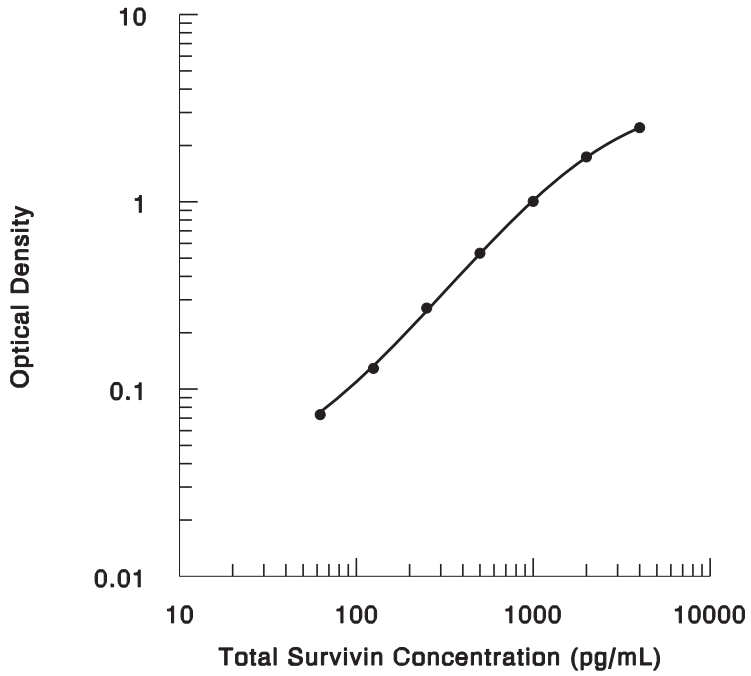
CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample, then subtract the average zero standard optical density (O.D.). Results may be normalized to total protein or cell number.

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 Survivin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

A standard curve should be generated for each set of samples assayed. The graph below represents typical data generated when using this Human Total Survivin DuoSet IC ELISA. The standard curve was calculated using a computer generated 4-PL curve-fit. This standard curve is for demonstration purposes only.



CALIBRATION

The Human Total Survivin DuoSet IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human Survivin produced at R&D Systems. Samples containing natural Survivin showed linear dilution parallel to the standard curve obtained using the Total Survivin Standard. These results indicate that O.D. values from this DuoSet IC ELISA can be used to determine the relative concentration of Survivin in natural samples.

SPECIFICITY

The Human Total Survivin DuoSet IC ELISA is specific for Survivin. Specificity was demonstrated by Western blot analysis of the protein bound by the capture antibody supplied in the kit.

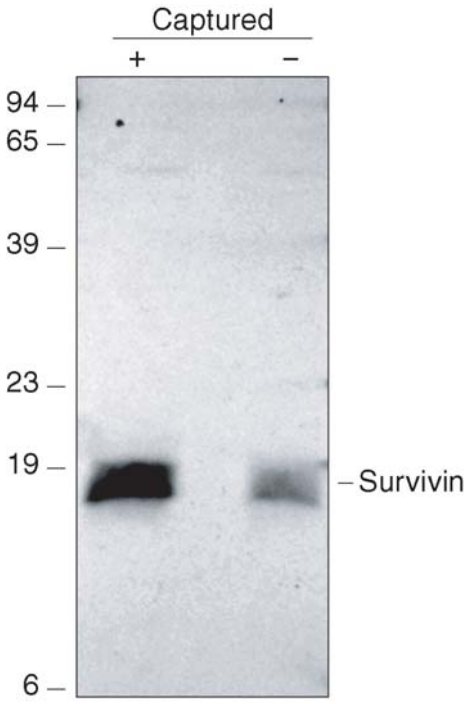


Figure 1: Lysates prepared from HepG2 human hepatocellular carcinoma cells, either untreated (-) or treated (+) with the G2/M blocking agent nocodazole, were incubated in wells coated with Human Total Survivin Capture Antibody. Unbound material was removed by washing and bound material was solubilized in SDS gel sample buffer. Captured proteins were electrophoresed, transferred to a PVDF membrane and immunoblotted with Human Total Survivin Detection Antibody. Only a single band corresponding to human Survivin was detected.

QUANTITATION

Absolute amounts of human Survivin, as measured by the Human Total Survivin DuoSet IC ELISA, are consistent with the relative amounts of human Survivin determined by qualitative Western blot analysis.

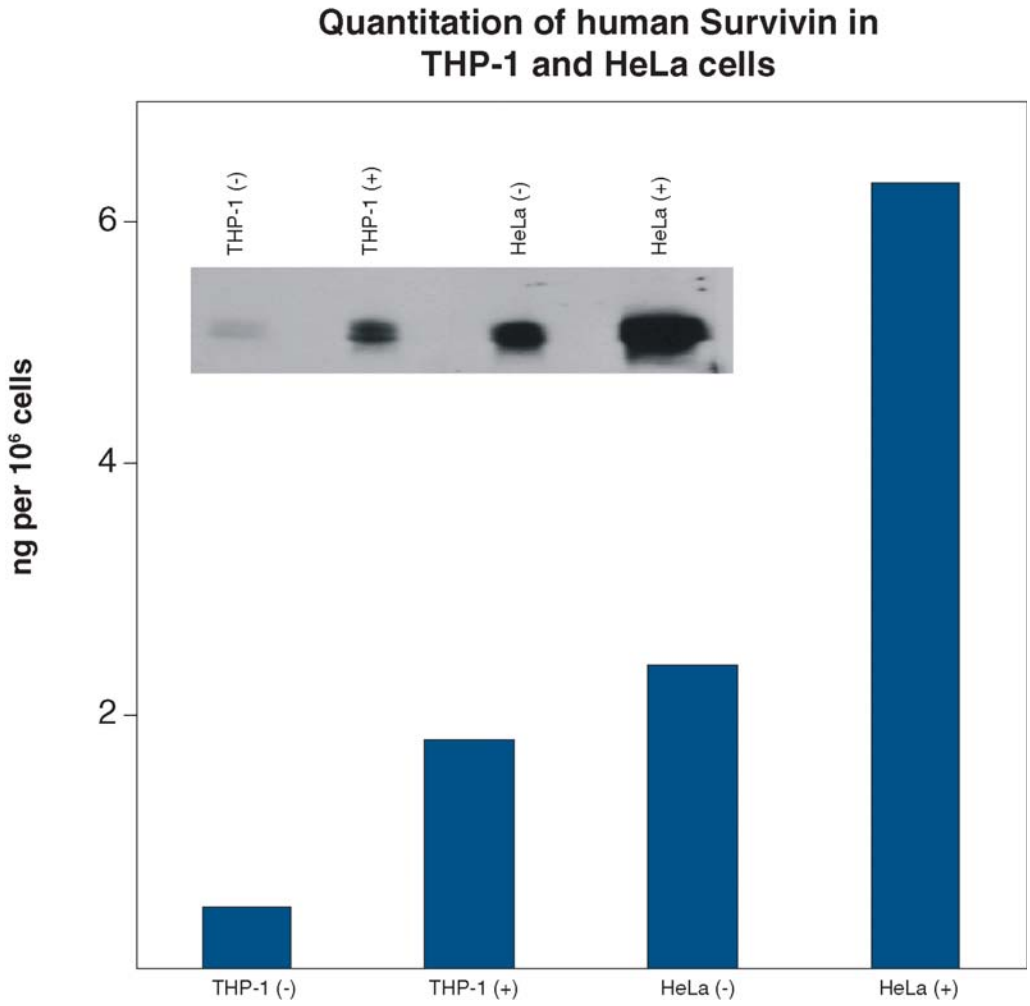
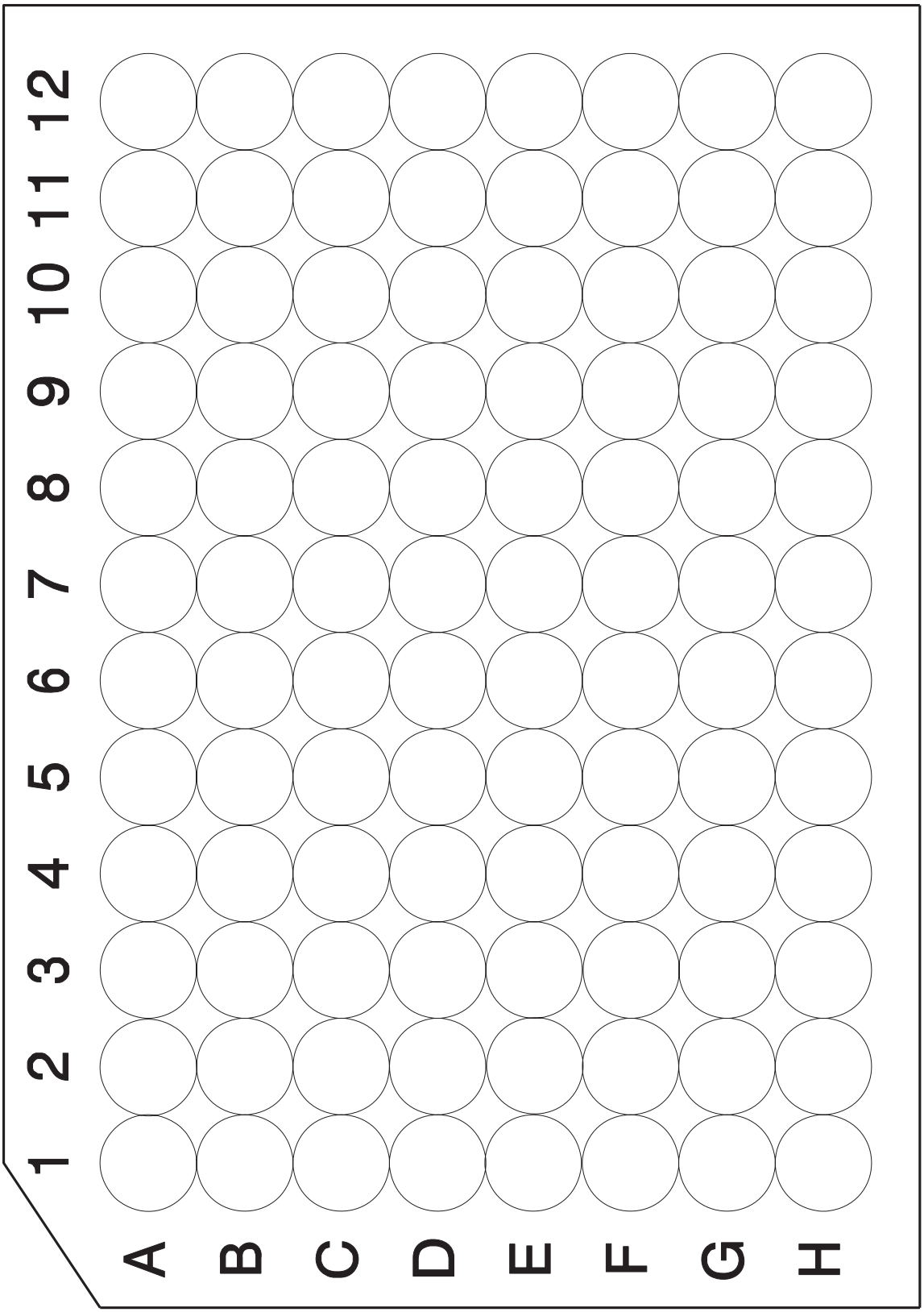


Figure 2: THP-1 human acute monocytic leukemia and HeLa human cervical epithelial carcinoma cells were either untreated (-) or treated (+) with 200 ng/mL nocodazole for 16 hours. Cells were lysed as described in the Preparation of Samples section on page 4. Human Survivin was measured with this DuoSet IC ELISA. The same lysates were immunoblotted (inset) with rabbit anti-human Survivin antibody (R&D Systems, Catalog # AF886). The DuoSet IC ELISA results correlate well with the relative amounts of Survivin detected by Western blot.

PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



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