# DuoSet® IC

## Human/Mouse/Rat Total SHP-2

Catalog Number DYC1894-2
DYC1894-5
DYC1894E

**For the development of sandwich ELISAs to measure Src Homology region 2 domain Phosphatase 2 (SHP-2) 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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## MANUFACTURED AND DISTRIBUTED BY:


R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com


## DISTRIBUTED BY:


R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

## PRINCIPLE OF THE ASSAY

This DuoSet® IC ELISA contains the basic components required for the development of ELISAs to measure Src Homology region 2 (SH2) domain Phosphatase 2 (SHP-2), also known as PTP1D, SH-PTP2, PTP2C, PTPN11, and Syp. An immobilized capture antibody specific for SHP-2 binds the protein. After washing away unbound material, a biotinylated secondary antibody specific for SHP-2 is used to detect bound SHP-2, utilizing a standard Steptavidin-HRP format.

## MATERIALS PROVIDED

**Store the unopened kit at 2-8° C. Do not use past kit expiration date.**

| | | | Vials Provided | |
|---|---|---|---|---|
| **Description** | **Part #** | **Storage Conditions** | **Cat. # DYC1894-2** | **Cat. # DYC1894-5** |
| Total SHP-2 Capture Antibody | 841917 | 2-8° C | 1 | 2 |
| Total SHP-2 Detection Antibody | 841918 | 2-8° C | 1 | 2 |
| Total SHP-2 Standard | 841919 | 2-8° C | 3 | 5 |
| Streptavidin-HRP | 890803 | 2-8° C | 1 | 1 |

DYC1894-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*
DYC1894-5 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 # DYC1894E). 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)
- NP-40 Alternative (EMD/Calbiochem # 492016)
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates [Costar EIA Plates (Catalog # 2592 or R&D Systems Catalog # DY990) are suggested]. For measurement of very low phosphatase activity, use 1/2 area microplates with a suitable plate reader [Costar EIA Plates (Catalog # 3690) are recommended]
- 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.

**TBS** - 25mM Tris, 150 mM NaCl, pH 7.5.

**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. (R&D Systems, Catalog # DY995).

**IC Diluent #10** - 50 mM HEPES, 0.1 mM EDTA, 0.1 mM EGTA, 0.5% NP-40 Alternative, 120 mM NaCl, pH 7.5. Store at 2-8° C.

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

**Lysis Buffer #8** - 50 mM HEPES, 0.1 mM EDTA, 0.1 mM EGTA, 120 mM NaCl, 0.5% NP-40 Alternative (pH 7.5), 25 μg/mL Leupeptin, 25 μg/mL Pepstatin, 2 μg/mL Aprotinin, 1 mM PMSF. Prepare fresh just before use.

**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 SHP-2 Capture Antibody** (Part 841917) - Each vial contains 360 μg/mL of rat anti-human SHP-2 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 SHP-2 Detection Antibody** (Part 841918) - Each vial contains 3.6 μg/mL of biotinylated rat anti-human SHP-2 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 SHP-2 Standard** (Part 841919) - Each vial contains 200 ng/mL of recombinant human SHP-2 when reconstituted with 500 μL of IC Diluent #10. **A fresh standard should be used for each assay. Use within one hour of reconstitution.** A seven point curve using 2-fold serial dilutions and a high standard of 10,000 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

**Note:** *Immediately before harvesting cells, prepare Lysis Buffer #8 and store it on ice until use.*

**Cell Lysates** - Rinse cells two times with TBS, making sure to remove any remaining PBS after the second rinse. Solubilize cells at 1 x $10^7$ cells/mL in Lysis Buffer #8 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 to a clean test tube. Sample protein concentration may be quantified using total protein assay. If needed, further dilutons should be made in IC Diluent #10.

## 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 diluent 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 a working concentration of 2 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 #10 per well. Use IC Diluent #10 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 10,000 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 100 ng/mL in IC Diluent #1 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.

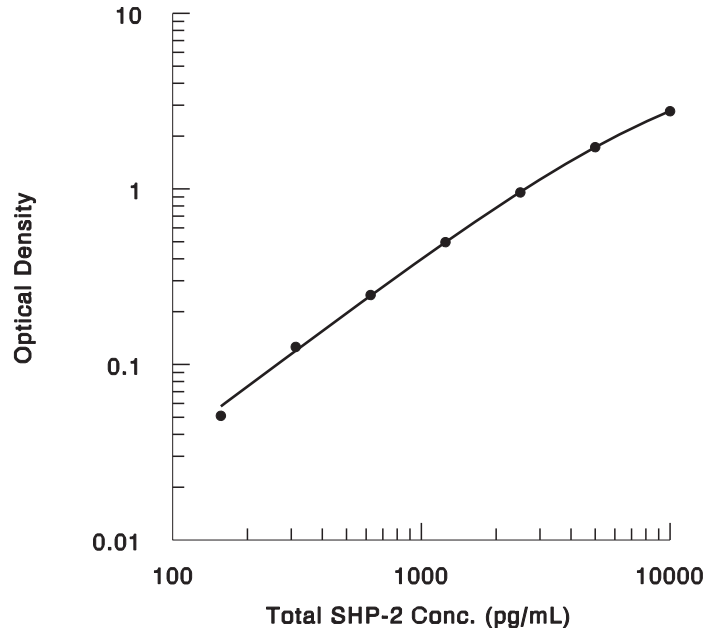
## CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample then subtract the average zero standard optical density. 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 SHP-2 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/Mouse/Rat Total SHP-2 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/Mouse/Rat Total SHP-2 DuoSet IC ELISA is calibrated against a highly purified *E. coli*-expressed recombinant human SHP-2 produced at R&D Systems. Samples containing natural SHP-2 showed linear dilution parallel to the standard curve obtained using the Total SHP-2 Standard. These results indicate that O.D. values from this DuoSet IC ELISA can be used to determine the concentration of SHP-2 in natural samples.

## SPECIFICITY

The Human/Mouse/Rat Total SHP-2 DuoSet IC ELISA specifically recognizes SHP-2. Specificity was demonstrated by Western blot analysis of protein immunoprecipitated with the capture antibody supplied in kit. One major band corresponding to SHP-2 was observed.

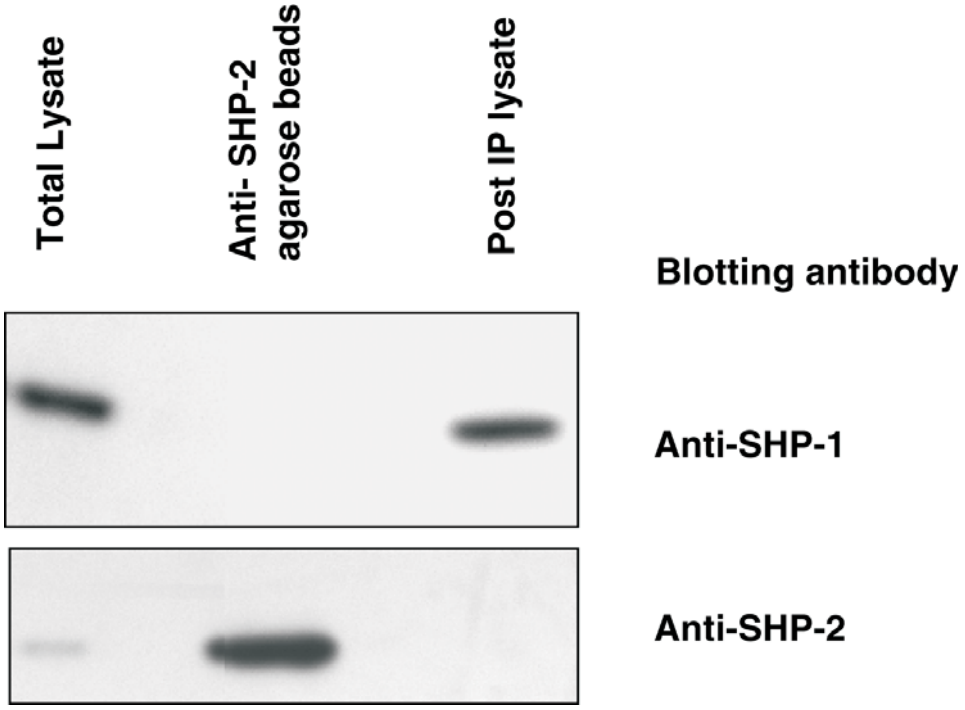



**Figure 1:** A lysate from Jurkat human acute T cell leukemia cells was immunoprecipitated with the Total SHP-2 Capture Antibody. Unbound material was removed by washing. The bound material was solublized in SDS-PAGE loading buffer and subjected to Western blotting using either anti-SHP-1 (R&D Systems, Catalog # AF1878) or anti-SHP-2 (R&D Systems, Catalog # AF1894) polyclonal antibodies. Both SHP-1 and SHP-2 were found in the original lysate but only SHP-2 was immunoprecipitated. SHP-1, which is 54% homologous to SHP-2, remained in the post-immunoprecipitation lysate.

## QUANTIFICATION

The amounts of human, mouse, and rat SHP-2 quantified by the Total SHP-2 DuoSet IC ELISA are consistent with the relative amounts of SHP-2 determined by Western blotting.

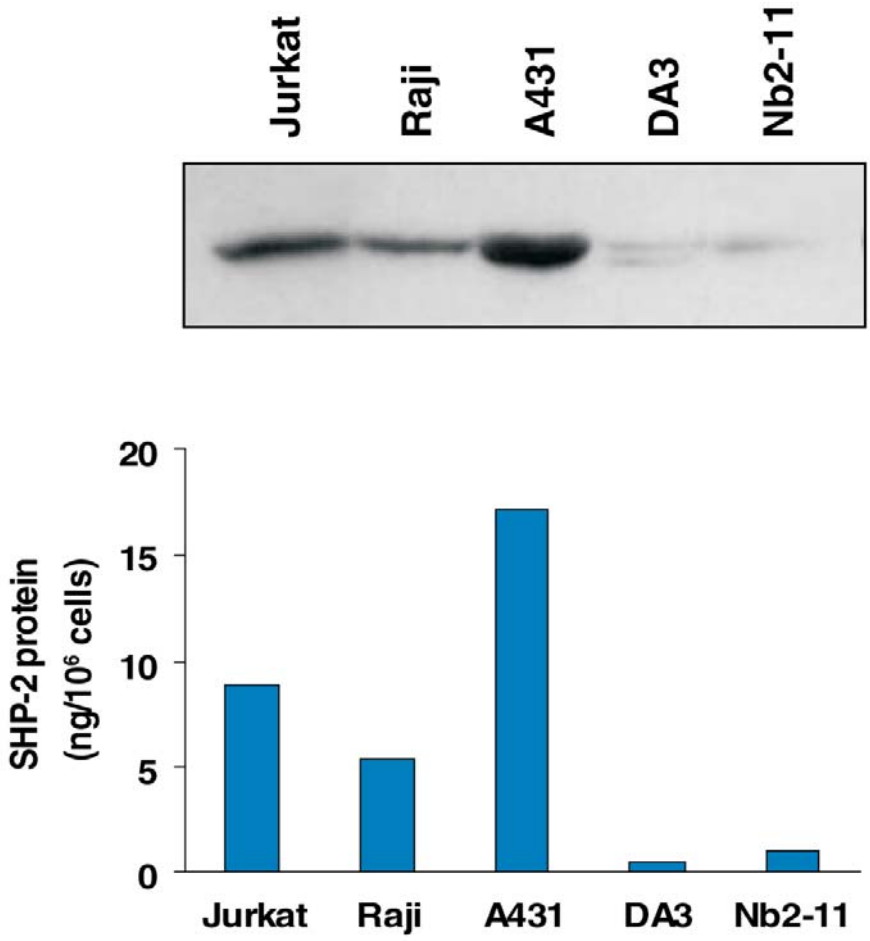
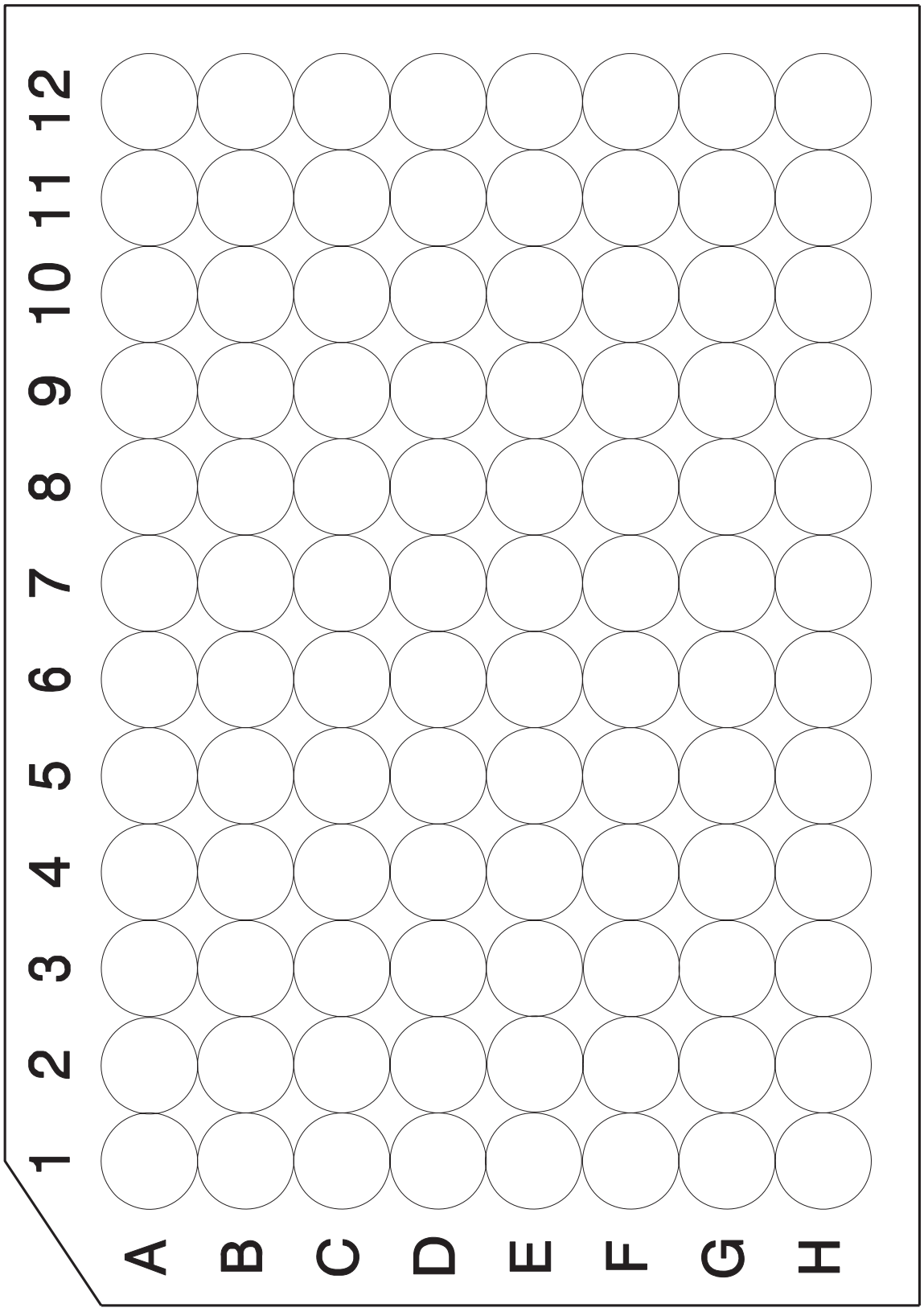



**Figure 2:** Lysates prepared from human Jurkat human acute T cell leukemia cells, Raji human Burkitt's lymphoma cells, A431 human epithelial carcinoma cells, DA3 mouse myleoma cells, and Nb2-11 rat lymphoma cells were quantified with this DuoSet IC ELISA. The same lysates were also immunoblotted with an anti-SHP-2 antibody (R&D Systems, Catalog # AF1894). The DuoSet IC ELISA results correlate well with the relative levels of SHP-2 detected by Western blot.

## PLATE LAYOUT

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



## NOTES