DuoSet® IC

Human Phospho-DDR1

Catalog Number DYC5859-2
DYC5859-5
DYC5859E

For the development of sandwich ELISAs to measure phosphorylated Discoidin Domain Receptor 1 (DDR1) 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.

TABLE OF CONTENTS

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 sandwich ELISAs to measure tyrosine-phosphorylated Discoidin Domain Receptor 1 (DDR1) in cell lysates. An immobilized capture antibody specific for human DDR1 binds both phosphorylated and unphosphorylated DDR1. After washing away unbound material, an HRP-conjugated monoclonal antibody specific for phosphorylated tyrosine is used to detect only phosphorylated receptor, utilizing a standard 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. # DYC5859-2**	**Cat. # DYC5859-5**
Human Phospho-DDR1 Capture Antibody	843443	2 - 8° C	1	2
anti-phospho-tyrosine-HRP	841403	2 - 8° C	1	2
Human Phospho-DDR1 Control	843445	2 - 8° C	3	5

DYC5859-2 contains sufficient materials to run ELISAs on at least two 96 well plates.*
DYC5859-5 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 # DYC5859E). 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)
- NP-40 Alternative (EMD/Calbiochem # 492016)
- Sodium azide (NaN_3) (Sigma # S2002)
- Sodium orthovanadate (Na_3VO_4) (Sigma # S6508), activated
- Pipettes and pipette tips
- Deionized or distilled water
- 96 well microplates (R&D Systems, Catalog # DY990)
- 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 (R&D Systems, Catalog # DY006).

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 #12** - 1% NP-40 Alternative, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate.

IC Diluent #14 - 20 mM Tris, 137 mM NaCl, 0.05% Tween 20, 0.1% BSA*, pH 7.2 - 7.4.

Lysis Buffer #9** - 1% NP-40 Alternative, 20 mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, 10 μg/mL Aprotinin, 10 μg/mL Leupeptin.

Note: *Lysis Buffer #9 consists of IC Diluent #12 plus 10 μg/mL Aprotinin and 10 μg/mL Leupeptin. Approximately 50 mL of IC Diluent #12 is required to run the assay on one 96 well plate.*

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.

**Alternately, use Sample Diluent Concentrate 2 (2X) (R&D Systems, Catalog # DYC002), prepared as described in the DYC002 insert.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Phospho-DDR1 Capture Antibody (Part 843443) - Each vial contains 180 μg/mL of goat anti-human DDR1 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.*

anti-phospho-tyrosine-HRP (Part 841403) - Each vial contains 50 μL of mouse anti-phospho-tyrosine antibody conjugated to HRP. Immediately before use, dilute the Detection Antibody to the working concentration specified on the vial label using IC Diluent #14. Prepare only as much anti-phospho-tyrosine-HRP as required to run each assay. Store at 2 - 8° C for up to 3 months after initial use.* **Do not freeze.**

Human Phospho-DDR1 Control (Part 843445) - Each vial contains 110 ng/mL of recombinant human phosphorylated DDR1 when reconstituted with 500 μL of IC Diluent #12. **Use within one hour of reconstitution. Use a fresh control for each assay.** A control concentration of 2000 pg/mL is recommended.

*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 #9 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 a total protein assay. If needed, further dilutions should be made in IC Diluent #12.

PRECAUTIONS

The Stop Solution recommended for use with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B recommended for use with this kit may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

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 sample and control reflect the environment of the samples being measured. The diluent suggested in this protocol should be suitable for most cell lysates.
- 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 controls 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 controls and samples assayed.

Plate Preparation

1. Dilute the Capture Antibody to a working concentration of 1.0 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 four times for a total of 5 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 control in IC Diluent #12 per well. Use IC Diluent #12 as the blank. Cover with a plate sealer and incubate 2 hours at room temperature.

   Note: *A control concentration of 2000 pg/mL is recommended.*
2. Repeat the aspiration/wash as in step 2 of Plate Preparation.
3. Add 100 μL of the diluted Anti-Phospho-Tyrosine-HRP to each well. Cover with a new plate sealer and incubate 2 hours at room temperature. Avoid placing the plate in direct light.
4. Repeat the aspiration/wash as in step 2 of Plate Preparation.
5. Add 100 μL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Add 50 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
7. 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 control and sample and then subtract the average blank optical density.

SENSITIVITY

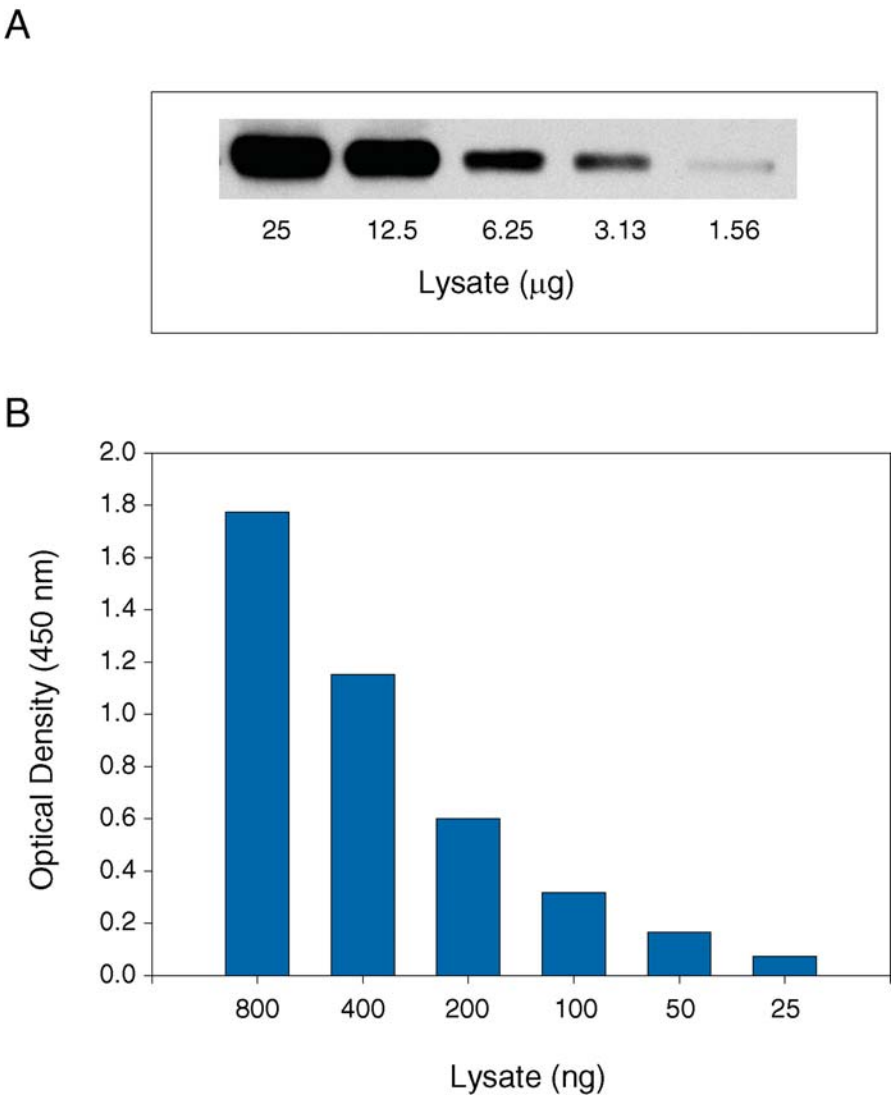


Figure 1: The Human Phospho-DDR1 DuoSet IC ELISA is more sensitive than immunoprecipitation (IP)-Western blot analysis. HEK293 cells transfected with human DDR1 (HEK293-hDDR1) were treated with 10 µg/mL of rat collagen I for 15 minutes to induce tyrosine phosphorylation of DDR1. Lysates were serially diluted and analyzed by **(A)** IP-Western blot and **(B)** this DuoSet IC ELISA. IPs were done using an anti-DDR1 polyclonal antibody and protein G agarose. Immunoblots were incubated with an HRP-conjugated anti-phospho-tyrosine monoclonal antibody (R&D Systems, Catalog # HAM1676) to detect phospho-DDR1. Bands were visualized by chemiluminescent detection. Human Phospho-DDR1 can be detected in this DuoSet IC ELISA by using approximately 10 - 20 times less lysate than is needed for a conventional IP-Western blot.

LIGAND-INDUCED PHOSPHORYLATION

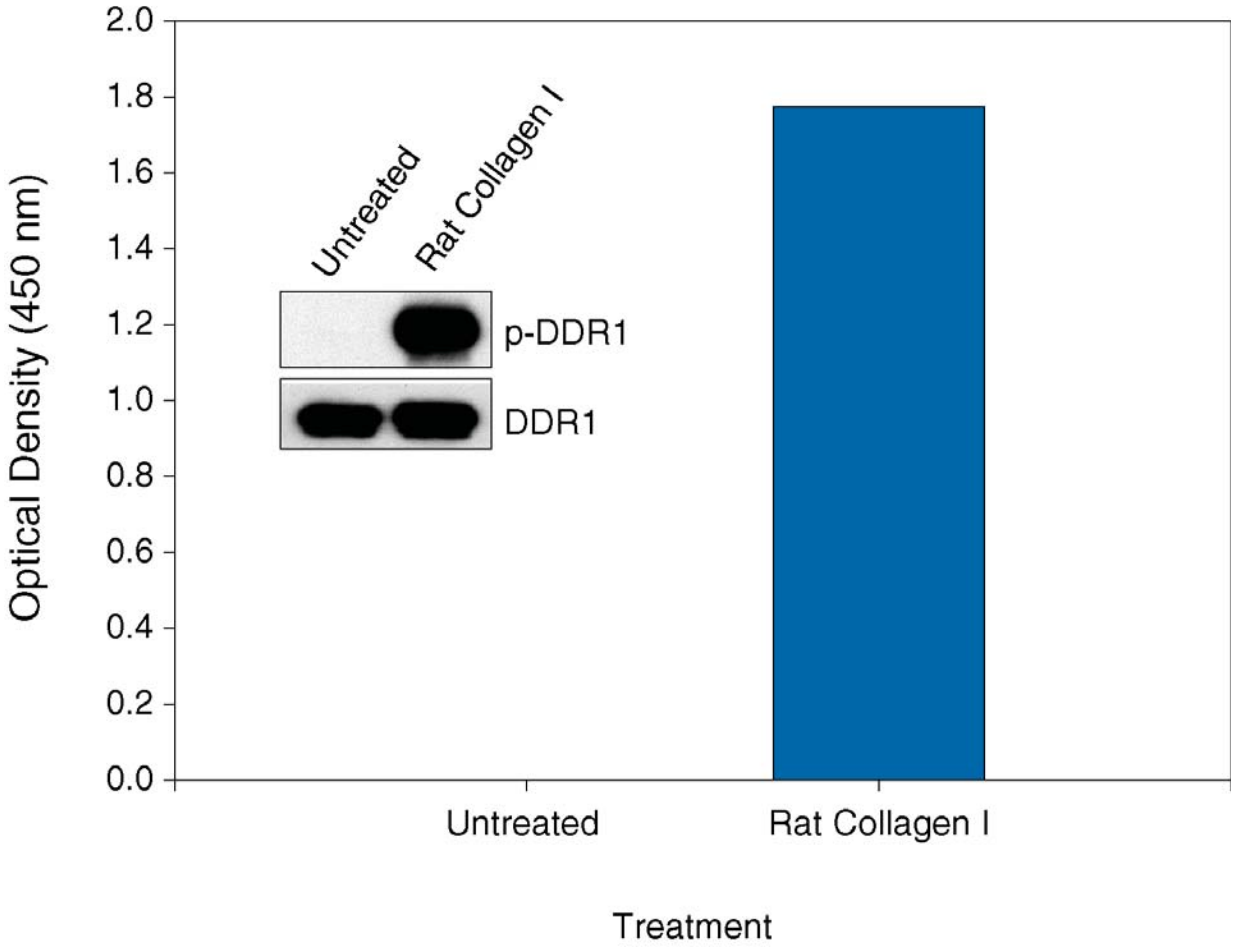


Figure 2: The Human Phospho-DDR1 DuoSet IC ELISA detects ligand-induced DDR1 tyrosine phosphorylation. HEK293-hDDR1 cells were untreated or treated with 10 μg/mL of rat collagen I for 15 minutes. ELISA and IP-Western blot (inset) analyses were done using 800 ng and 25 μg of lysate, respectively. IP-Western blots for phospho-DDR1 (p-DDR1) were done as described in Figure 1. Blots were stripped and total DDR1 was detected using a biotinylated anti-DDR1 polyclonal antibody.

SPECIFICITY

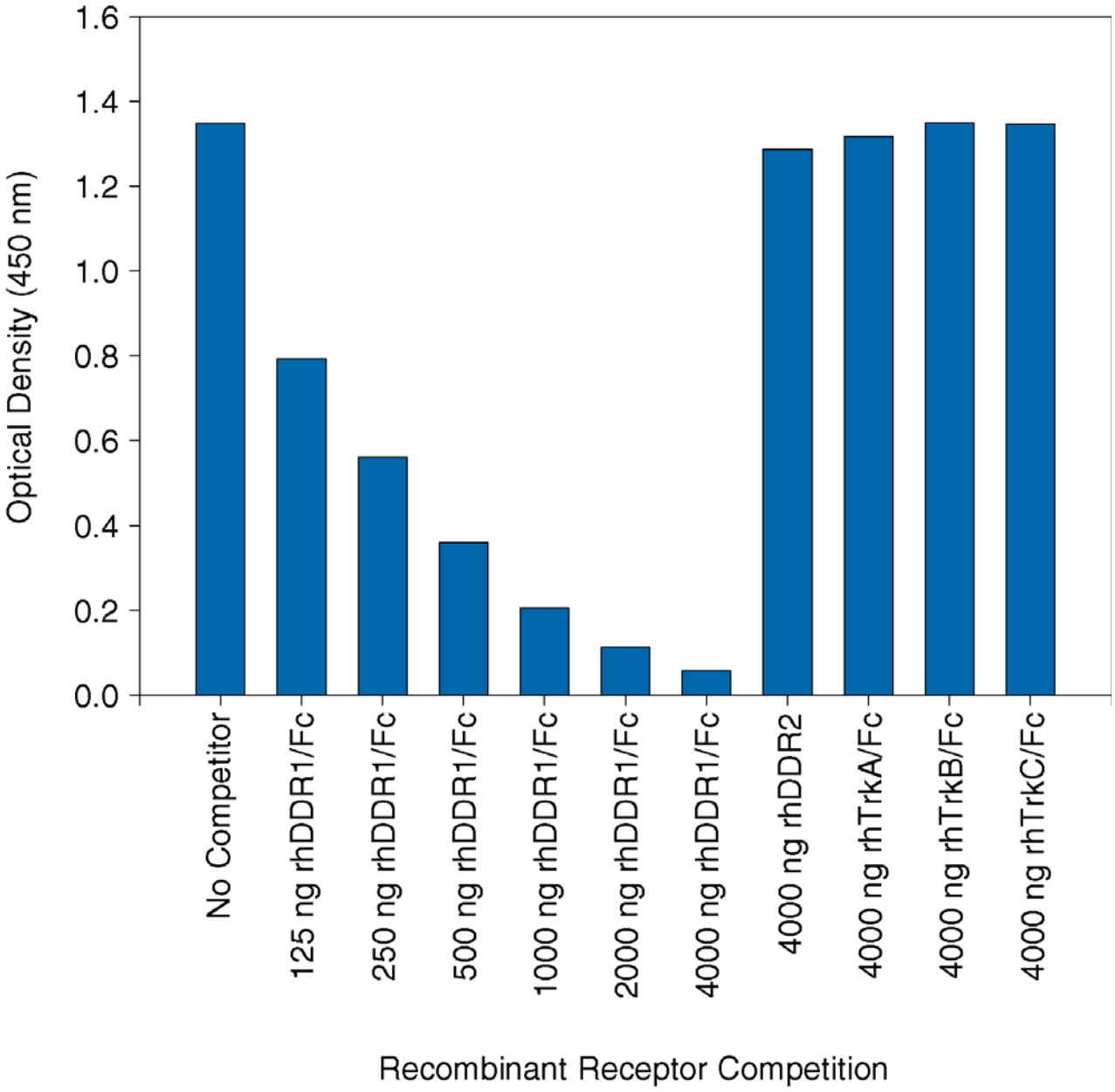
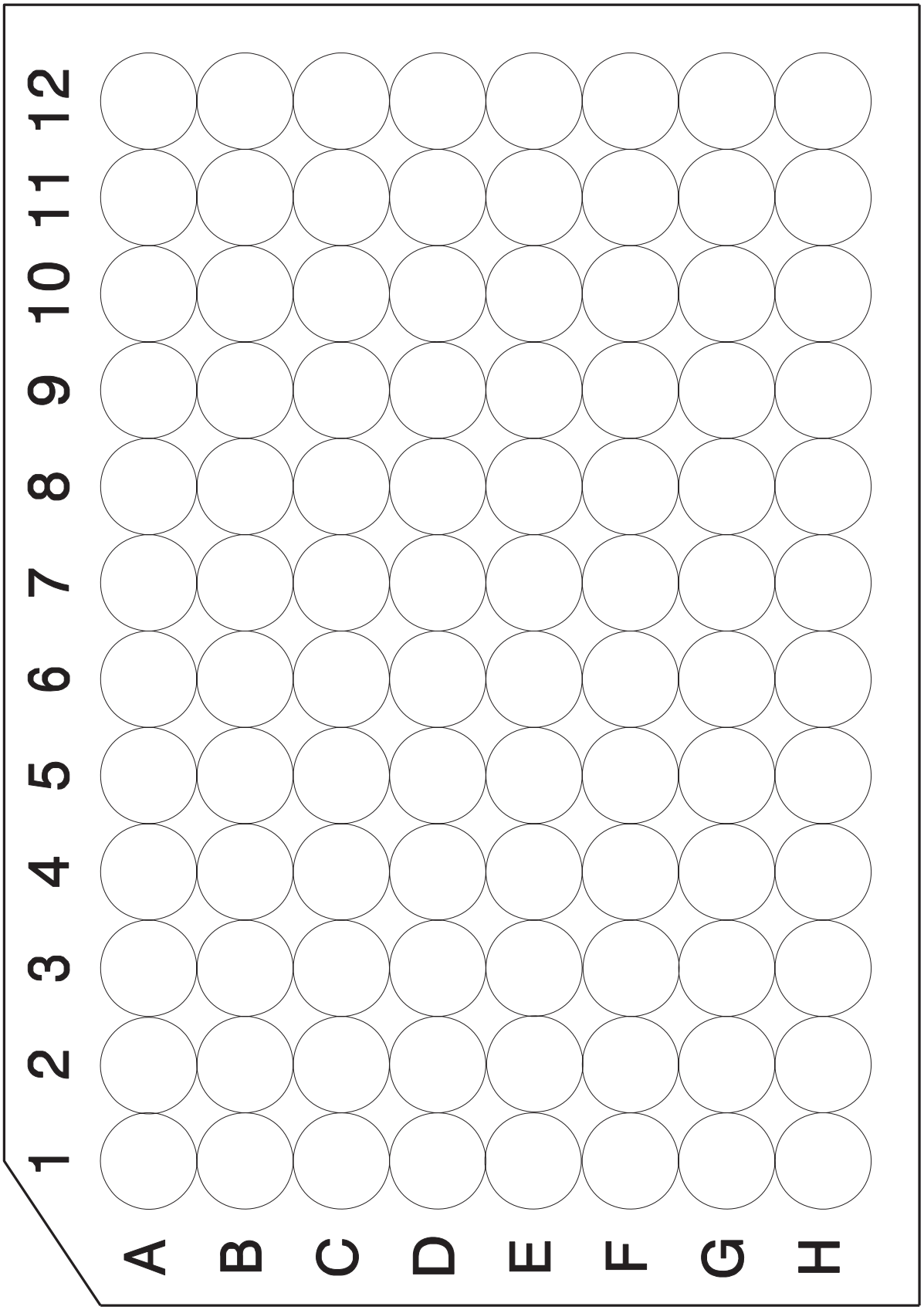


Figure 3: The specificity of the Human Phospho-DDR1 DuoSet IC ELISA is confirmed by receptor competition. HEK293-hDDR1 cells were treated with 10 μg/mL of rat collagen I for 15 minutes. The indicated amounts of recombinant extracellular domains of human DDR1/Fc Chimera (R&D Systems, Catalog # 2396-DR), human DDR2 (R&D Systems, Catalog # 2538-DR), human TrkA/Fc Chimera (R&D Systems, Catalog # 175-TK), human TrkB/Fc Chimera (R&D Systems, Catalog # 688-TK), or human TrkC/Fc Chimera (R&D Systems, Catalog # 373-TC) were added to 800 ng of lysate and analyzed using this DuoSet IC ELISA. Competition was observed only with recombinant human DDR1.

PLATE LAYOUT

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



NOTES