MSD MULTI-ARRAY® Assay System

Phospho-PERK (Thr980) Kit

1-Plate Kit	K150OED-1
5-Plate Kit	K150OED-2
25-Plate Kit	K150OED-4

MSD® Phosphoprotein Assays

Phospho-PERK (Thr980) Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

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Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Protein kinase-like endoplasmic reticulum kinase (PERK) is a transmembrane protein kinase of the endoplasmic reticulum (ER) that activates itself by oligomerization and autophosphorylation in response to ER stress and the accumulation of mis-folded proteins in the ER lumen. Phosphorylation of PERK occurs at Threonine 981 (Thr981) in humans (Thr980 in rats) and further enhances PERK activity.[1] PERK phosphorylates the eukaryotic translation initiation factor 2 alpha (eIF2α) and the Nrf2 transcription factor. Phosphorylation of eIF2α results in attenuation of translation initiation and contributes to cell cycle arrest due to loss of the G1 regulatory protein, cyclin D1.[2] PERK-dependent phosphorylation of Nrf2 promotes transcription of detoxifying enzymes, which is critically important in the regulation of genes involved in metabolism, the redox status of the cells, and apoptosis.[2,3] The ER is also the major subcellular compartment involved in calcium storage, lipid production, and protein biosynthesis in which a variety of extracellular signaling molecules and protein receptors critical for cellular homeostasis are properly folded, assembled, matured, and transported to their destination. However, folding activity can be overwhelmed under ER stress, during which unfolded proteins accumulate in the ER and trigger downstream signaling pathways called the unfolded protein response (UPR).[2-4] PERK is an important component of the UPR-mediated adaptation pathways to ER stress. Chronic activation of PERK can induce cell death, and increasing evidence indicates that ER stress and PERK activation are associated with a variety of diseases, including diabetes, neurodegenerative diseases, cancer, bipolar disease, liver diseases, cardiac diseases, muscle degeneration, and autoimmune diseases.[1-5]

Principle of the Assay

MSD phosphoprotein assays provide a rapid and convenient method for measuring biomarkers within a single, small-volume sample. Phospho-PERK (Thr980) is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures intensity of emitted light to provide a quantitative measure of analytes in the sample.

1. **pPERK**
2. *BSA Blocked*
3. *BSA Blocked*
4. *BSA Blocked*

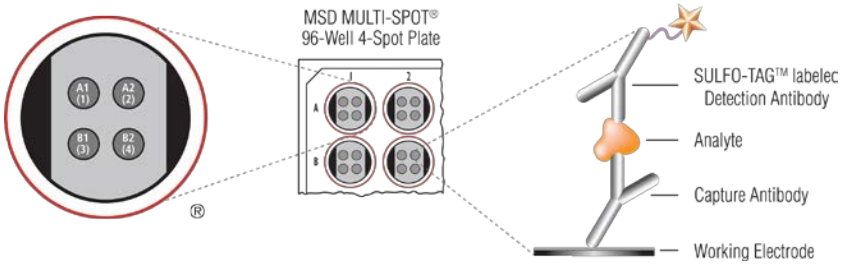


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K1500ED-1	K1500ED-2	K1500ED-4
MULTI-SPOT 96-Well 4-Spot PERK Plate N450NIA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-Phospho-PERK (Thr980) Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL)
Tris Lysis Buffer (1X) R60TX-3 (50 mL)	2–8°C	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Tris Wash Buffer (10X) R61TX-2 (200 mL)	2–8°C	1 bottle (200 mL)	1 bottle (200 mL)	5 bottles (200 mL ea)
Phosphatase Inhibitor I (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	5 vials (0.5 mL ea)
Phosphatase Inhibitor II (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	5 vial (0.5 mL ea)
Protease Inhibitor Solution (100X)	2–8°C	1 vial (0.1 mL)	1 vial (0.5 mL)	5 vials (0.5 mL ea)
Blocker D-R[2] (10%)	≤-10°C	1 vial (0.05 mL)	1 vial (0.2 mL)	5 vials (0.2 mL ea)
Blocker A (dry powder) R93BA-4	RT	1 vial (15 g)	1 vial (15 g)	5 vials (15 g ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Materials and Equipment (not supplied)

- Appropriately sized tubes and bottles for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker
- Deionized water

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.
[2] Blocker D-R can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of Blocker D-R can be stored at 2-8°C for up to 1 month.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Reagent Preparation

Prepare Tris Wash Buffer

Dilute the 10X stock of Tris Wash Buffer provided to 1X as shown below. Tris Wash Buffer (1X) will be used throughout the assay to make additional reagents and wash plates. Approximately 350 mL per plate is required—more if using an automatic plate washer.

For 1 plate, combine:

- ❑ 35 mL of Tris Wash Buffer (10X)
- ❑ 315 mL of deionized water

Excess Tris Wash Buffer may be stored at room temperature in a tightly sealed container.

Prepare Blocking Solution

For 1 plate, combine:

- ❑ 600 mg of Blocker A (dry powder)
- ❑ 20 mL of 1X Tris Wash Buffer

Prepare Antibody Dilution Buffer

For 1 plate, combine:

- ❑ 1 mL of blocking solution
- ❑ 1.97 mL of 1X Tris Wash Buffer
- ❑ 30 µL of 10% Blocker D-R

Set aside on ice.

Prepare Complete Lysis Buffer

Prepare complete lysis buffer just prior to use. The working solution is 1X.

For 1 plate, combine:

- ❑ 50 µL of Protease Inhibitor Solution (100X stock)
- ❑ 50 µL of Phosphatase Inhibitor Solution I (100X stock)
- ❑ 50 µL of Phosphatase Inhibitor Solution II (100X stock)
- ❑ 4.85 mL of 1X Tris Lysis Buffer

Immediately place the complete lysis buffer on ice; it should be ice cold before use.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-Phospho-PERK (Thr980) Antibody
- ❑ 2.94 mL of cold antibody dilution buffer

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL Read Buffer T (4X)
- ❑ 15 mL deionized water

You may prepare diluted 1X read buffer in advance and store it room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (see Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Sample Preparation and Storage

We developed the Phospho-PERK (Thr980) assay using cell lysates from rat hepatoma cell line that was modified to overexpress PERK protein. The assay has been optimized for quantifying phosphorylated PERK at threonine 980 in the cytosolic fraction of the lysate.

Most lysis buffers and sample matrices are compatible with MSD plates, although high concentrations of denaturing reagents should be avoided. Keep SDS and other ionic detergents to a concentration of 0.1% or less in the sample applied to the well and avoid reducing agents (DTT >1mM). Please contact MSD Scientific Support if you have any questions about lysate preparation options.

Analysis of proteins in their activated state (i.e. phosphorylated) usually requires stimulation prior to cell lysis. Verify cell stimulation and sample preparation prior to using this kit.

Perform all manipulations on ice; keep PBS wash buffer and complete lysis buffer ice cold. Cell concentrations for lysis can range from 0.5 to 5 x 10^7 cells per mL of lysis buffer. Protein yields will vary by cell line. To get your desired final protein concentration, you will need to optimize the number of cells used and the amount of complete lysis buffer added. Depending on the stability of the target in the matrix, you may need additional protease and phosphatase inhibitors in the matrix or diluent.

MSD provides suggested cell lysis protocols in the appendix; however, specific cell types or targets may benefit from alternative buffer components or techniques, depending upon the particular research application.

Assay Protocol

1. **Block Plate and Prepare Samples:**
   a. Add 150 µL of blocking solution to each well. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   b. Prepare complete lysis buffer just prior to sample dilution. See the Sample Preparation and Storage section above for recommended sample handling procedures.
   c. Prepare positive and negative cell lysates.
      - Thaw cell lysate samples on ice and dilute them immediately before use. Keep on ice during all manipulations and discard all unused, thawed, material.
      - Dilute cell lysate in complete lysis buffer to a final concentration of 0.8 µg/µL. This will deliver 20 µg of lysate in 25 µL per well. You may prepare a dilution series at this point if desired.

2. **Wash and Add Samples:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 25 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal, and incubate for 3 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 25 µL of detection antibody solution to each well of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare 1X read buffer during incubation.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of Tris Wash Buffer. Add 150 µL of 1X Read Buffer T to each well of the MSD plate.

   Analyze the plate on the SECTOR Imager. No incubation in read buffer is required.

Notes

Shaking the plate typically accelerates capture at the working electrode.

Solutions containing MSD Blocker A should be stored at 2-8°C and discarded after 14 days.

Complete lysis buffer should be kept ice-cold during all experiment manipulations.

If working with purified protein, only a few nanograms per well will generally provide a strong assay signal. Purified recombinant proteins may exhibit differences in both signal and background as compared to native proteins in cell lysates.

Samples, including cell lysates, may be used neat or diluted.

It is not possible to prepare serial dilutions in the MSD plate. Use microcentrifuge tubes or a separate 96-well polypropylene plate to prepare serial dilutions.

The sensitivity of MSD immunoassays usually exceeds ELISAs and Western blots. The amount of sample required for a given assay will depend on the abundance of the analyte in the matrix and the affinities of the antibodies used.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

Due to the varying nature of each research application, you should investigate assay stability before allowing plates to sit with read buffer for extended periods.

Assay Components

The capture and detection antibodies used in this assay are listed below. They cross-react with rat and mouse cell lysates.

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Phospho-PERK (Thr980)	Rabbit Polyclonal	Goat Polyclonal

References

1. Parmar VM, Schröder M. Sensing endoplasmic reticulum stress. Adv Exp Med Biol. 2012;738:153-68
2. Fels DR, Koumenis C. The PERK/eIF2alpha/ATF4 module of the UPR in hypoxia resistance and tumor growth. Cancer Biol Ther. 2006 Jul;5(7):723-8.
3. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol. 2012 Jan 18;13(2):89-102.
4. Rath E, Haller D. Inflammation and cellular stress: a mechanistic link between immune-mediated and metabolically driven pathologies. Eur J Nutr. 2011 Jun;50(4):219-33.
5. Ozcan U, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science. 2004 Oct 15;306(5695):457-61.

Appendix: Suggested Cell Lysis Protocols

Preparation in Culture Flask or Petri Dish

Suspension Cells. Pellet cells by centrifugation at 500 x g for 3 minutes at 2–8°C. Discard supernatant and wash the pellet once with cold PBS. Pellet cells again, discard supernatant, and resuspend in complete lysis buffer at 1–5 x 10^7 cells per mL. Incubate on ice for 30 minutes. (A shorter incubation time of 15 minutes may be adequate for many targets.) Clear cellular debris from the lysate by centrifuging (≥10 000 x g) for 10 minutes at 2–8°C. Discard the pellet and determine the protein concentration in the lysate using a detergent-compatible protein assay such as a bicinchoninic acid (BCA) assay. Unused lysates should be aliquoted, quickly frozen in a dry ice-ethanol bath, and stored at ≤-70°C.

Adherent Cells. All volumes given are for cells plated on 15 cm dishes. Remove media from the dish and wash cells one time with 5 mL cold PBS. Add 2 mL PBS to each dish, scrape the cells from the surface of the dish, and transfer into 15 mL conical tubes. Pellet the cells by centrifugation at 500 x g for 3 minutes at 2–8°C. Discard supernatant and resuspend cells in 0.5–2 mL of complete lysis buffer per dish. (Alternatively, cells can be lysed by adding 1–2 mL of complete lysis buffer per 15 cm dish after completely removing the PBS wash buffer. Cell lysate can be collected by snapping the dish surface prior to the clarifying spin.) Incubate on ice for 30 minutes. A shorter incubation time of 15 minutes may be adequate for many targets. Clear cellular debris from the lysate by centrifuging (≤10 000 x g) for 10 minutes at 2–8°C. Discard the pellet and determine protein concentration in the lysate using a detergent compatible protein assay such as BCA. Unused lysates should be aliquoted, quickly frozen in a dry ice-ethanol bath, and stored at ≤-70°C.

Preparation in 96-well Culture Plate

Successful adaptation to a 96-well culture format depends on cell type and target. First, determine the number of cells of each cell type to be plated per well. MSD generally recommends plating concentrations ranging from 1 x 10^4 to 10^5 cells per well; however, the optimal concentrations will vary depending on cell line used.

Suspension Cells. You may lyse many cell types without removing growth medium. For flat bottom plates, design the experiment so that the final suspension cell volume per well is such that a concentrated complete lysis buffer (prepared by the user) can be added to the well to achieve a final a 1X lysis buffer concentration in the well. For example, 40 µL of 5X complete lysis buffer added to a well containing 160 µL of cell culture medium would provide a 1X concentration of complete lysis buffer.

For conical microwell plates, perform lysis by pelleting the cells, removing most of the growth medium, and adding a constant amount of 1X complete lysis buffer.

Adherent Cells. Plate cells on coated tissue culture plates to reduce variability due to cells lost as growth medium is removed. Treat cells as desired. Gently aspirate growth medium from the microwell plate to avoid disrupting the cell monolayer. A PBS wash step is not required and can introduce variability as cells may detach during the wash step. Add 100 µL of 1X complete lysis buffer per well. You may modify lysis volume for different cell types or applications.

You will need to determine the optimum cell lysis time. Some targets are immediately available for detection. Other targets may require an incubation step at room temperature or on ice with gentle agitation.

Carefully pipet cell lysate onto prepared plate and proceed with assay protocol. Note: It is important to transfer a constant volume and to avoid introducing air bubbles by pipetting too vigorously.

Summary Protocol

Phospho-PERK (Thr980) Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Phospho-PERK (Thr980) assay.

Reagent Preparation

Prepare Tris Wash Buffer.

Prepare blocking solution.

Prepare antibody dilution buffer.

Prepare detection antibody solution by diluting 50X detection antibody 50-fold in antibody dilution buffer.

Prepare 1X Read Buffer T by diluting 4X Read Buffer T 4-fold with deionized water.

Step 1: Block Plate and Prepare Samples

Add 150 µL/well of blocking solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Prepare complete lysis buffer just prior to sample dilution.

Prepare positive and negative cell lysates and keep on ice until use.

Step 2: Wash and Add Sample

Wash the plate 3 times with 300 µL/well of Tris Wash Buffer.

Add 25 µL/well of sample (standards, controls, or unknowns).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 3 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash the plate 3 times with 300 µL/well of Tris Wash Buffer.

Add 25 µL/well of 1X detection antibody solution.

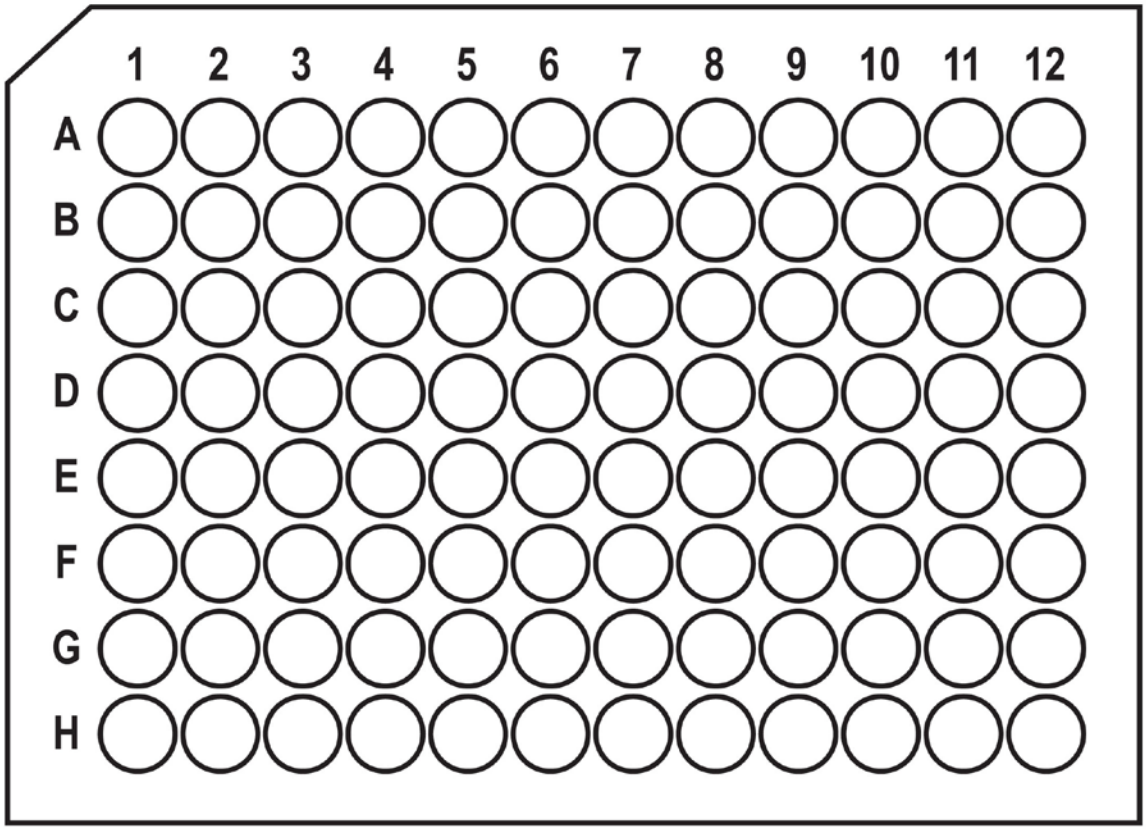
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash the plate 3 times with 300 µL/well of Tris Wash Buffer.

Add 150 µL/well of 1X Read Buffer T.

Analyze plate on SECTOR Imager within 5 minutes of adding read buffer.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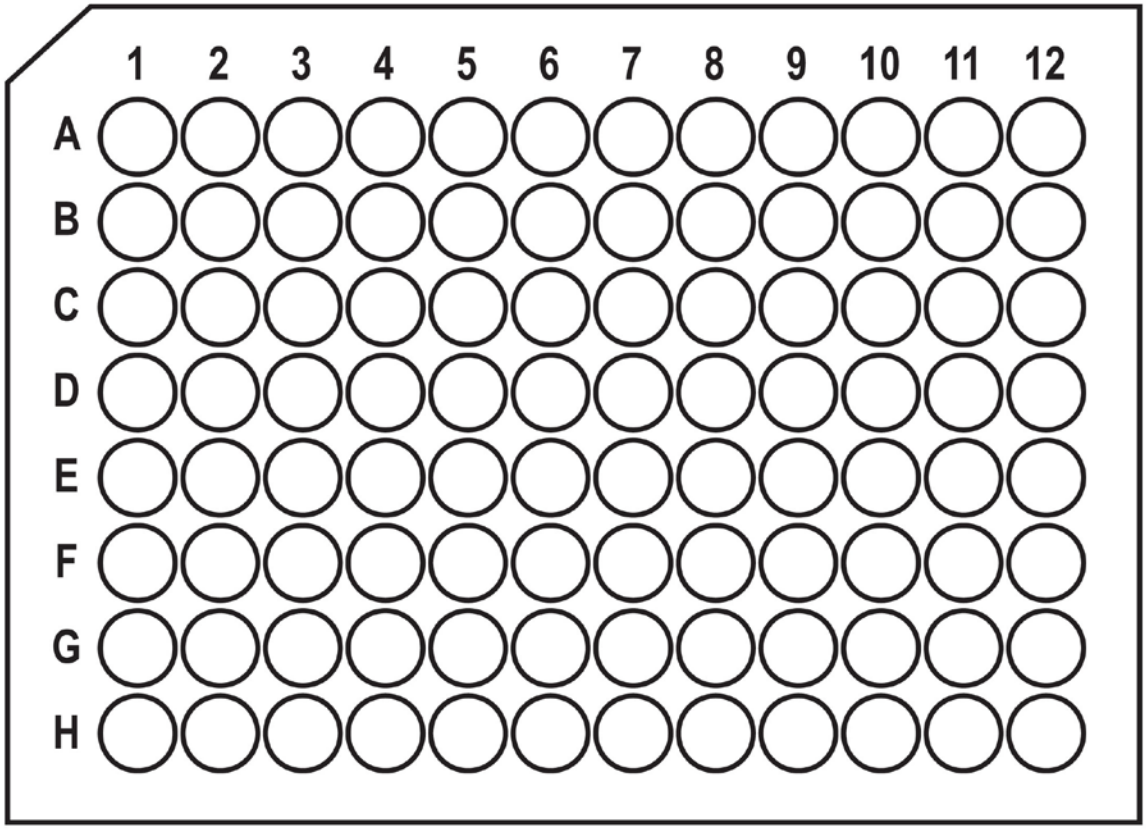
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