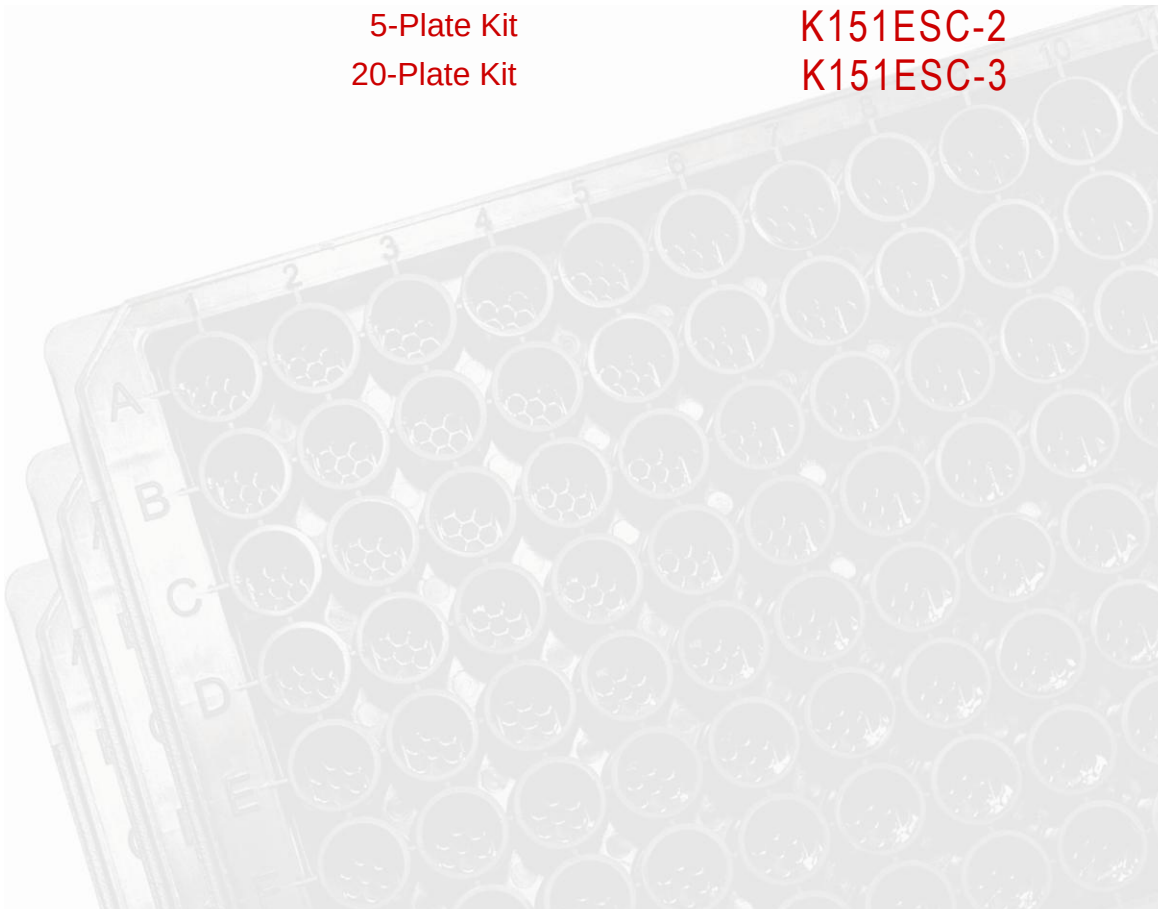
MESO SCALE DISCOVERY

MULTI-ARRAY Assay System

Human FSH Assay Kit

1-Plate Kit	K151ESC-1
5-Plate Kit	K151ESC-2
20-Plate Kit	K151ESC-3



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MSD Fertility Assays

Human FSH Assay

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

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

introduction

Follicle stimulating hormone (FSH) is a highly heterogeneous glycoprotein that plays a central role in mammalian reproduction. It is necessary for reproductive activities of the gonadal tissues, maturation of the gonads at puberty and for gamete production. It is essential for folliculogenesis in the female and spermatogenesis in the male. The activity of FSH is regulated by the levels of the reproductive hormones through a negative feedback system. FSH is secreted by the basophil cells of the pituitary gland, and this is controlled by the gonadotropin-releasing hormone (1-4).

Similar to other hormones, FSH consists of two polypeptide chains, α and β. These chains contain carbohydrate moities N-linked to Asparagine residues. The sugar part of FSH consists of fucose, galactose, mannose, galactosamine, glucosamine, and sialic acid. All the glycoprotein hormones have a common 92 amino acid long α-subunit; thus, the biological properties of each are dependent on the unique beta subunit. The beta subunit of FSH has 118 amino acid residues. FSH has a half-life of 3 – 4 hours (5, 6)

FSH acts by binding to its specific receptor, FSH-R. This belongs to the family of G-protein coupled, seven transmembrane protein receptors. Activation of FSH-R initiates a cascade of events that generates the biological effects of FSH. In males, FSH-R is expressed in Sertoli cells, whereas, in females, it is expressed in granulosa cells (7-9).

High levels of FSH are observed after menopause and in premature ovarian failure. Polycystic ovarian disease has also been associated with abnormal FSH levels. High levels of FSH have been observed in renal failure, cirrhosis and hyperthyroidism. In men, elevated FSH concentrations have been found in Kleinfelter syndrome, testicular failure, and in azospermic and oligospermic individuals (1, 2, 4).

Principle of the Assay

principle of the assay

MSD® fertility assays provide a rapid and convenient method for measuring the levels of protein targets within a single small-volume sample. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Human FSH Assay detects FSH in a sandwich immunoassay format (Figure 1). MSD provides a plate that has been pre-coated with FSH antibody. The user adds the sample and a solution containing the labeled detection antibody— Anti-hFSH labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. FSH in the sample binds to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of FSH present in the sample.

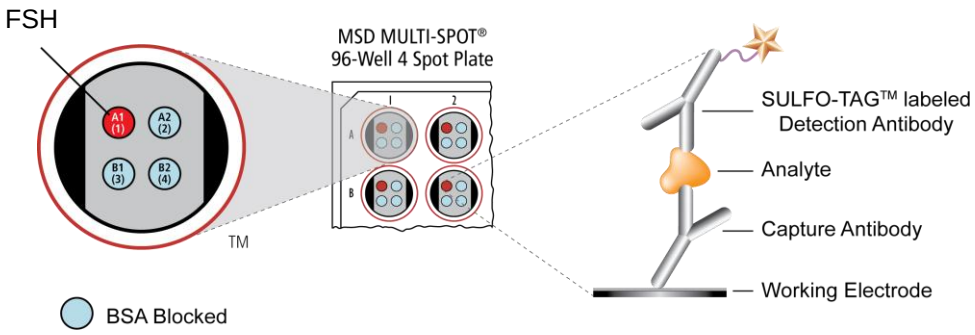


Figure 1. *Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K151ESC-1	K151ESC-2	K151ESC-3
MULTI-SPOT 96-well 4 Spot Human FSH Plate N451ESA-1	2–8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-hFSH Detection Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	4 vials (375 µL ea)
Human FSH Calibrator[2] (160 mIU/mL)	≤-70°C	1 vial (125 µL)	5 vials (125 µL ea)	20 vials (125 µL ea)
Diluent 23 R50BC-8 (1.5 mL) R50BC-4 (15 mL)	≤-10°C	1 bottle (1.5 mL)	1 bottle (15 mL)	4 bottles (15 mL ea)
Diluent 22 R50BB-8 (8 mL) R50BB-4 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	2 bottles (40 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL ea)

IV Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate 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

V Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

[2] The Calibrator in this kit is derived from human source material which has been tested and found to be negative for HIV-1 and 2, Hepatitis B, and Hepatitis C. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

> **Important:** Upon first thaw, separate Diluent 22 and Diluent 23 into aliquots appropriate to the size of your assay needs. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Calibrator and Control Solutions

Calibrator for the Human FSH Assay is supplied at the concentration of the highest Calibrator. For the assay, an 8-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Human FSH Calibrator (mIU/mL)	Dilution Factor
STD-01	160	
STD-02	40	4
STD-03	10	4
STD-04	2.5	4
STD-05	0.63	4
STD-06	0.16	4
STD-07	0.039	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 3 replicates:

1) Calibrator for the Human FSH Assay is supplied at the concentration of the highest Calibrator. Therefore, no dilution is required for top of the curve.
2) Prepare the next Calibrator by transferring 30 µL of the undiluted Calibrator to 90 µL of Diluent 23. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 90 µL of Diluent 23 to be used as the 8th (zero) calibrator.

Notes:

a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.

b. The standard curve can be modified as necessary to meet specific assay requirements.

Dilution of Samples

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 50X stock of Anti-hFSH Antibody. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock Detection Antibody solution into 2.94 mL of Diluent 22.

Prepare Read Buffer

The Read Buffer should be diluted 4-fold in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of 4X Read Buffer T to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

Notes

1. **Addition of Detection Antibody Solution:** Dispense 25 µL of the 1X Detection Antibody Solution into each well of the MSD plate.
2. **Addition of Sample or Calibrator:** Dispense 25 µL of sample or Calibrator into separate wells 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.

Shaking a 96-well MSD plate typically accelerates capture at the working electrode.

3. **Wash and Read:** Wash the plate 3 times with PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Bubbles in the fluid will interfere with reliable reading of plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

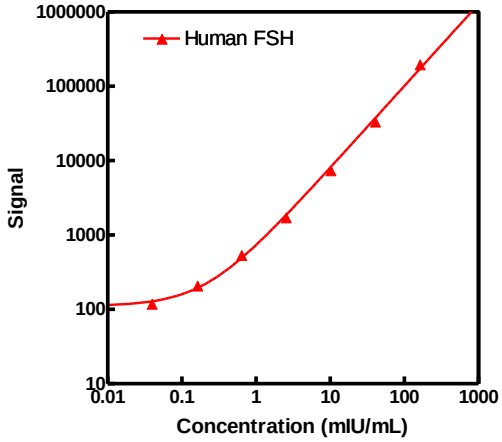
analysis of results

The Calibrator should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



FSH		
Conc. (mIU/mL)	**Average Signal**	**%CV**
0	105	2.0
0.039	120	8.4
0.16	211	0.7
0.63	544	8.9
2.5	1740	2.1
10	7464	5.6
40	33808	4.9
160	199109	4.4

X Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator. The value below represents the average LLOD over multiple kit lots.

	FSH
LLOD (mIU/mL)	0.050

XI Assay Components

Assay components

The human FSH capture and detection antibodies used in this assay are listed below.

	Source species	
Analyte	**MSD Capture Antibody**	**MSD Detection Antibody**
hFSH	Mouse monoclonal	Mouse monoclonal

XIII References

references

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2. Chappel SC. Heterogeneity of follicle stimulating hormone: control and physiological function. Hum Reprod Update. 1995 Sep;1(5):479-87.
3. Moyle, W.R. and Campbell, R.K. Gonadotropins, In Endocrinology, ed. L.J. de Groot, W.B. Saunders Co., Philadelphia pp230-241. (1994)
4. Simoni M, Nieschlag E. FSH in therapy: physiological basis, new preparations and clinical use. Reprod Med Rev. 1995 4:163–177
5. Parsons TF, Strickland TW, Pierce JG. Disassembly and assembly of glycoprotein hormones. Methods Enzymol. 1985 109:736–749
6. Ulloa-Aguirre A, Midgley AR Jr, Beitins IZ, Padmanabhan V. Follicle-stimulating isohormones: characterization and physiological relevance. Endocr Rev. 1995 Dec;16(6):765-87.
7. Sprengel R, Braun T, Nikolics K, Segaloff DL, Seeburg PH. The testicular receptor for follicle stimulating hormone: structure and functional expression of cloned cDNA. Mol Endocrinol. 1990 Apr;4(4):525-30.
8. Gudermann T, Nürnberg B, Schultz G. Receptors and G proteins as primary components of transmembrane signal transduction. Part 1. G-protein-coupled receptors: structure and function. J Mol Med. 1995 Feb;73(2):51-63.
9. Chappel S, Buckler D, Kelton C, Tayar NE. Follicle stimulating hormone and its receptor: future perspectives. Hum Reprod. 1998 Jun;13 Suppl 3:18-35; discussion 47-51.

Summary Protocol

MSD 96-well MULTI-ARRAY® Human FSH Assay

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Human FSH Assay.

Step 1: Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Prepare an 8-point standard curve using supplied calibrator and conducting 7-fold dilution in Diluent 23. Use Diluent 23 as zero calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-hFSH Antibody to 1X in a final volume of 3.0 mL of Diluent 22 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 2: Add Detection Antibody Solution

Dispense 25 µL/well 1X Detection Antibody Solution.

Step 3: Add Sample or Calibrator

Dispense 25 µL/well Calibrator or sample.

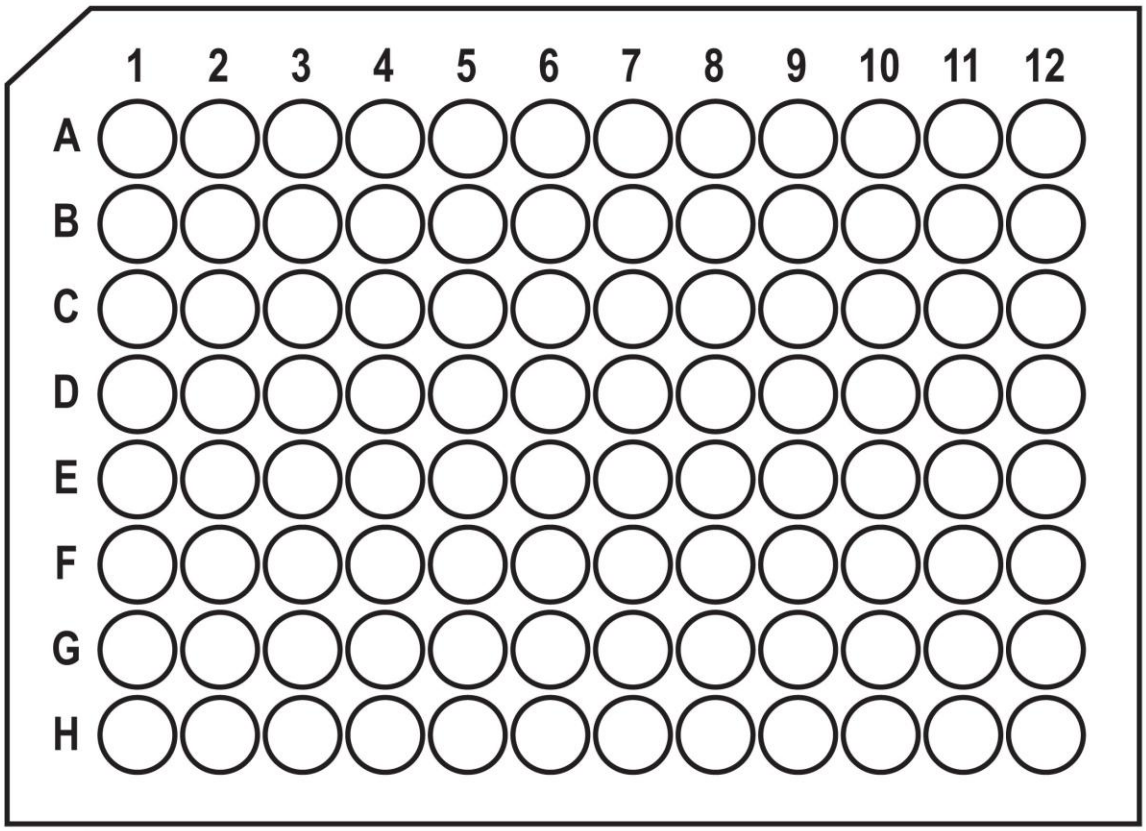
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 1X Read Buffer T.

Analyze plate on SECTOR Imager instrument.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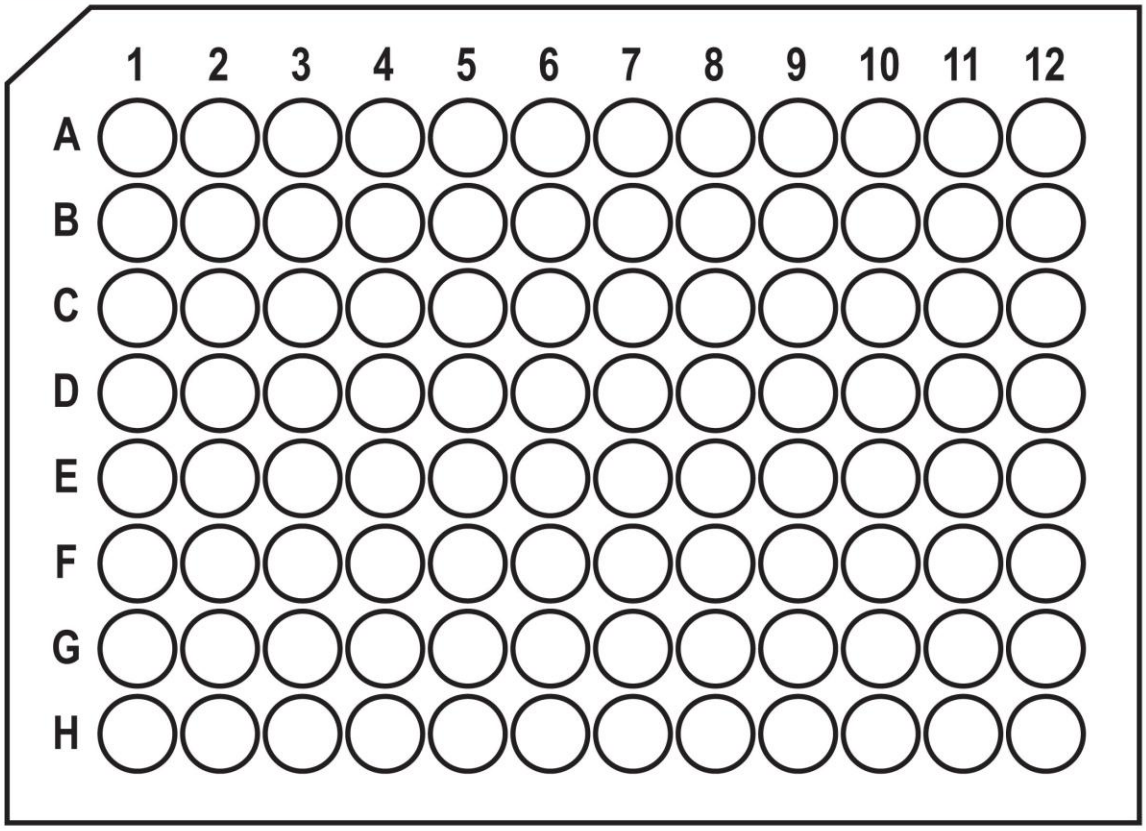
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