MSD® MULTI-ARRAY Assay System

Rat B2M Assay Kit

1-Plate Kit	K153KAC-1
5-Plate Kit	K153KAC-2
25-Plate Kit	K153KAC-4

www.mesoscale.com®

MSD Toxicology Assays

Rat B2M Assay Kit

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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A division of Meso Scale Diagnostics, LLC.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

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Ordering Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Beta-2-Microglobulin (B2M) is an 11.8 kDa secreted and ubiquitously expressed protein that is involved in the presentation of peptide antigens to the immune system. Mature rat B2M is a 99-amino acid polypeptide containing IgC beta2m region (24-116) corresponding to class 1 major histocompatibility complex (MHC). B2M is normally filtered in the kidneys through the glomerulus, reabsorbed, and metabolized in the cells of the proximal tubules.[1] Under normal conditions, trace amounts of B2M are excreted in the urine. The levels of B2M rise in the event of kidney toxicity associated with glomerular injury and/or renal tubular damage.[2-4] Elevated serum B2M levels have also been reported in conditions involving reduced Glomerular Filtration Rate (GFR) and are reportedly an indicator of allograft rejection in patients undergoing kidney transplant.[5,6]

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Rat B2M assay has been qualified according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al.[7] The Rat B2M Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with streptavidin, and the user adds rat B2M capture antibody conjugated to biotin. The user then adds the sample and a solution containing the detection antibody conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Biotin-conjugated capture antibody binds to the streptavidin that has been immobilized on the working electrode surface, analyte in the sample binds to the capture antibody, and the recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument 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 B2M present in the sample.

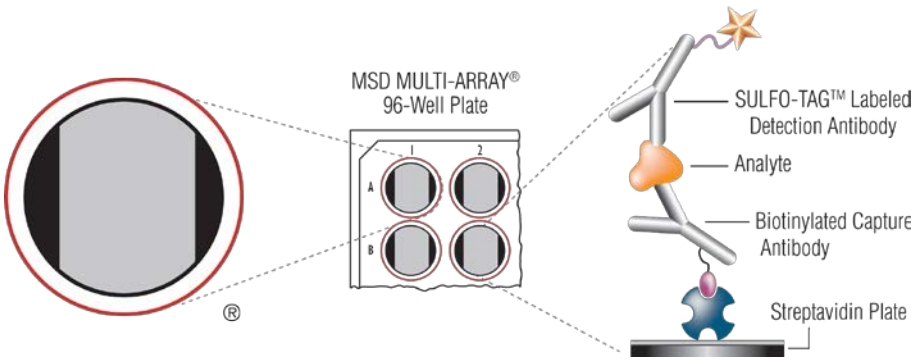


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K153KAC-1	K153KAC-2	K153KAC-4
MULTI-ARRAY 96-Well Streptavidin Gold Plate L15SA-1	2–8°C	1 plate	5 plates	25 plates
Anti-rat B2M Biotinylated Capture Antibody (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
SULFO-TAG Anti-rat B2M Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat B2M Calibrator (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 5 R52BA-5 (25 mL)	≤-10°C	1 bottle (25 mL)	4 bottles (25 mL ea)	20 bottles (25 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well 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.

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. Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 5 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 Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Capture Antibody Solution

The biotin-conjugated capture antibody is provided as a 50X stock solution. The final concentration of the working capture antibody solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock capture antibody into 2940 µL of Diluent 5.

Prepare Calibrator Dilutions

Calibrator for the Rat B2M Assay is supplied at 20-fold higher concentration than the recommended highest calibrator. An 8-point standard curve is recommended with 4-fold serial dilution steps and a zero calibrator. The stock calibrator should be thawed and kept on ice and then should be added into diluent at room temperature to make the standard curve solutions. For the actual concentration of the calibrator, refer to the certificate of analysis (COA) supplied with the kit. A copy of the kit-specific COA can also be found at www.mesoscale.com

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

1) Prepare the highest calibrator by adding 10 µL of the calibrator stock vial to 190 µL of Diluent 5. Mix well.
2) Prepare the next calibrator by transferring 60 µL of the diluted calibrator to 180 µL of Diluent 5. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) The recommended 8th standard is Diluent 5 (i.e. zero calibrator).

Calibrators should be prepared at room temperature no more than 20 minutes before use.

Dilution of Samples

Rat urine samples should be diluted prior to the assay in order to get the analyte levels into the detection range. A 1000-fold dilution of urine samples is recommended for this assay. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

Samples should be prepared in two dilution steps as follows:

1) Add 10 µL of sample to 490 µL of 1X PBS (50-fold dilution)
2) Add 10 µL of the 50-fold diluted sample into 190 µL of Diluent 5 (20-fold dilution)

If a different sample dilution factor is desired, step 1 should be modified accordingly with no change to step 2. Rat urine samples should always be diluted by at least 20-fold prior to the assay.

Diluted samples should be prepared at room temperature no more than 20 minutes before use.

Prepare Detection Antibody Solution

The detection antibody is provided as a 50X stock solution. 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 into 2940 µL of Diluent 5.

Prepare Read Buffer

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

Prepare MSD Plate

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.

Assay Protocol

Notes

1. **Addition of Blocker A Solution:** Dispense 150 µL of Blocker A solution into 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.

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

2. **Wash and Addition of Capture Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 1X capture antibody solution into each well of the MSD plate. Seal the plate, and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

3. **Addition of the Sample or Calibrator:** Without washing the plate, dispense 25 µL of calibrator or diluted sample into separate wells of the MSD plate. Seal the plate with an adhesive plate seal, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

4. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 1X detection antibody solution into each well of the MSD plate. Seal the plate, and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

5. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on an MSD instrument. No incubation in read buffer is required before reading the plate.

Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to ensure bubbles are not created when dispensing the read buffer.

Analysis of Results

The calibrators should be run minimally 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 quantification 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.

Assay Validation and Verification

The performance of this kit meets levels of consistency and robustness as determined by methods based on the principles outlined in "Fit -for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al.[7]

Bioanalytical and functional characterizations of calibrators, antibodies, and assay components are completed to allow for bridging of reagents between lots. This includes plate coating uniformity and reagent and component specificity testing for individual kit lots.

Control samples for specific matrices are designed and tested to meet the accuracy, precision, and sensitivity criteria for a kit that has completed the validation process. Spike recovery and dilution linearity of endogenous samples, pooled and individual matrices, are tested across the assay range.

- **Sensitivity, Range, and Curve Fitting**
  - Sample range and assay sensitivity are established from 4-parameter logistic fitted calibration curves with $1/Y^2$ weighting. Percent recovery of calibrators and controls between the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) must have calculated concentration %CV of less than 20% and accuracy within 20% of the expected concentration.
  - The limits of quantification defined in the product insert are verified for each lot as part of the lot verification and quality control release.

- **Accuracy and Precision**

High, mid, and low controls made in matrix (defined on a kit-by-kit basis) are run to measure accuracy and precision.

  - Validation – The assay is tested over multiple days (>6 days) and multiple runs per day for a total of 15-20 runs of complete kits. Precision is measured for the standard curve for intra- and inter-day coefficients of variance (CVs) of less than 20%. The typical specification includes a calculated concentration CV of less than 20%, accuracy within 20% of expected concentration, and a total error of less than 30%. The kit specifications for this lot are provided in the enclosed COA.
  - Verification – A multi-day (2-3 days) analysis with multiple runs per day of 6-12 total plates is performed as part of the release testing for each lot. The specifications for release are provided in the COA.

- **Robustness and Stability**

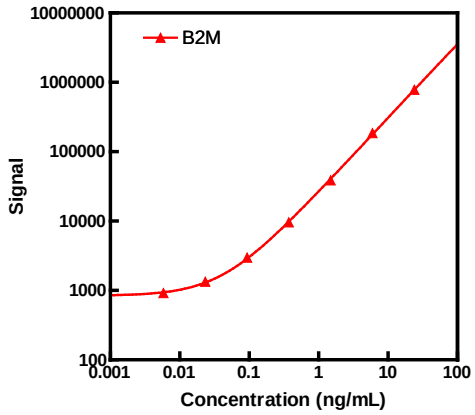
Freeze-thaw testing and accelerated stability studies performed during assay development (calibrators, antibodies, controls) are augmented with real-time stability studies on complete kits out to 18 months from the date of manufacture.

All acceptance criteria and verification conformance are defined in the COA for all kit lots. Presented below are representative data from the assay validation for this assay that meets the criteria described above. The kit lot-specific standard curve and measured limits of quantification can be found in the COA enclosed with the kit.

Typical Data

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 quantification of unknown samples.

Some variation in the concentration of the highest calibrator is permissible between kit lots. Below is a table that details the acceptable range of the highest calibrator concentration. For each individual kit lot, the calibrator concentrations are shown in the COA.



	B2M	
Conc. (ng/mL)	Average Signal	%CV
0	759	4.7
0.00583	915	4.8
0.0233	1338	3.8
0.0934	2973	5.2
0.373	9605	2.3
1.49	38 714	4.6
5.98	185 089	3.6
23.9	780 993	2.5

Sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the blank (zero calibrator).

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

	B2M (ng/mL)
LLOD	0.00500
LLOQ	0.0488
ULOQ	20.0

Precision

Control samples of high, mid, and low levels were measured on each plate.

Controls were made as follows:

High control: 275-fold diluted normal rat urine in Diluent 5

Mid control: High control samples diluted 4-fold in Diluent 5

Low control: Mid control samples diluted 4-fold in Diluent 5

All three controls were run neat.

The controls were run in quadruplicate on each of 3 plates run across multiple days (n>3).

Average intra-plate %CV is the average %CV of the control replicates within an individual plate.

Inter-plate %CV is the variability of controls across 9 plates over 3 days.

Inter-lot %CV is the variability of controls across 2 kit lots.

	Control	Plates	Average Conc. (ng/mL)	Average Intra-plate %CV	Inter-plate %CV	Inter-lot %CV
B2M	High	16	4.61	3.1	3.9	4.3
	Mid	16	0.981	2.6	4.3	4.3
	Low	16	0.245	3.3	6.5	5.5

Spike Recovery

Normal rat urine was spiked with the calibrator at multiple levels throughout the range of the assay. The samples were diluted 1000-fold (as shown in the Dilution of Samples section) and then spiked with calibrator at the levels indicated in the table below.

% Recovery = measured / expected x 100

	B2M			
Sample	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Urine	0	0.877	4.5	
	0.488	1.34	3.3	98
	1.95	2.89	2.9	102
	7.81	9.55	3.0	110

Linearity

To assess linearity, urine samples were diluted 500-fold, 1000-fold, 2000-fold, and 4000-fold prior to testing. The diluted samples were prepared as follows:

1) 25-fold dilution: 10 µL of sample in 240 µL of 1X PBS
2) 20-fold dilution: 10 µL of the 25-fold diluted sample in 190 µL of Diluent 5 (500-fold dilution)
3) 2-fold dilution: 100 µL of 500-fold diluted sample in 100 µL of Diluent 5 (1000-fold dilution)
4) Repeat 2-fold serial dilutions 2 additional times to generate 2000 and 4000-fold dilutions.

The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration measured from the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

		B2M		
Sample	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Urine	500	963	2.3	
	1000	890	2.6	92
	2000	867	2.6	97
	4000	842	4.1	97

Samples

Urine samples collected from normal Sprague-Dawley rats were tested at 1000-fold dilution in the Rat B2M Assay. Shown below are the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution.

Sample	Statistic	B2M
Urine (male rat)	Median (ng/mL)	1004
	Range (ng/mL)	606-2036
	N	20
Urine (female rat)	Median (ng/mL)	365
	Range (ng/mL)	155-953
	N	20

Assay Components

Calibrator

Recombinant rat B2M protein was used as calibrator for the Rat B2M Assay.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
B2M	Goat Polyclonal	Goat Polyclonal

References

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Summary Protocol

MSD 96-well MULTI-ARRAY Rat B2M Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Rat B2M Assay.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature, and thaw the calibrator on ice.
- ❑ Prepare capture antibody solution by diluting the 50X capture antibody to 1X in a final volume of 3.0 mL Diluent 5 per plate.
- ❑ Prepare an 8-point standard curve using the supplied calibrator:
  - The calibrator should be diluted in Diluent 5.
  - Dilute the stock calibrator 20-fold in Diluent 5. Then perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples by 50-fold into 1XPBS followed by 20-fold dilution into Diluent 5 prior to addition to the plate.
- ❑ Prepare detection antibody solution by diluting the 50X detection antibody to 1X in a final volume of 3.0 mL Diluent 5 per plate.
- ❑ Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 1 : Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2 : Wash and Add Capture Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X capture antibody solution into each well of the MSD plate.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Add Sample or Calibrator

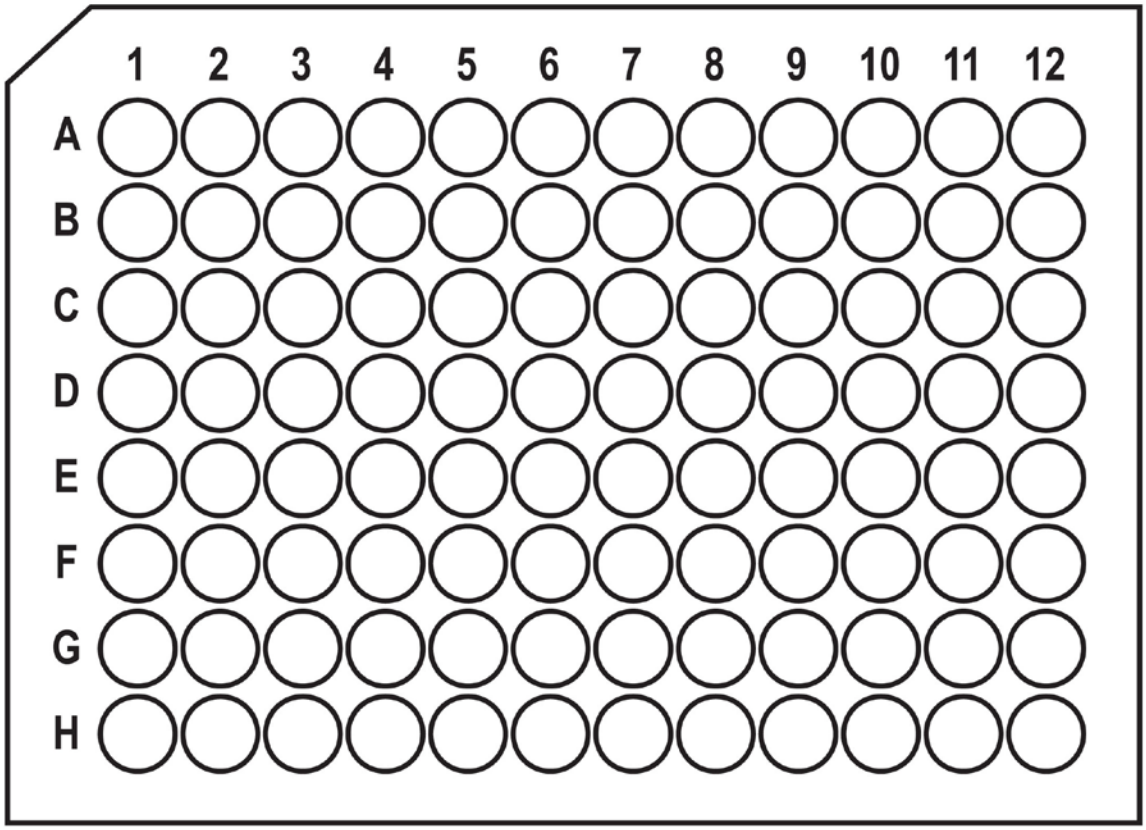
- ❑ Do not wash the plate.
- ❑ Add 25 µL/well of calibrator or diluted sample.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 5 : Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 5 : Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.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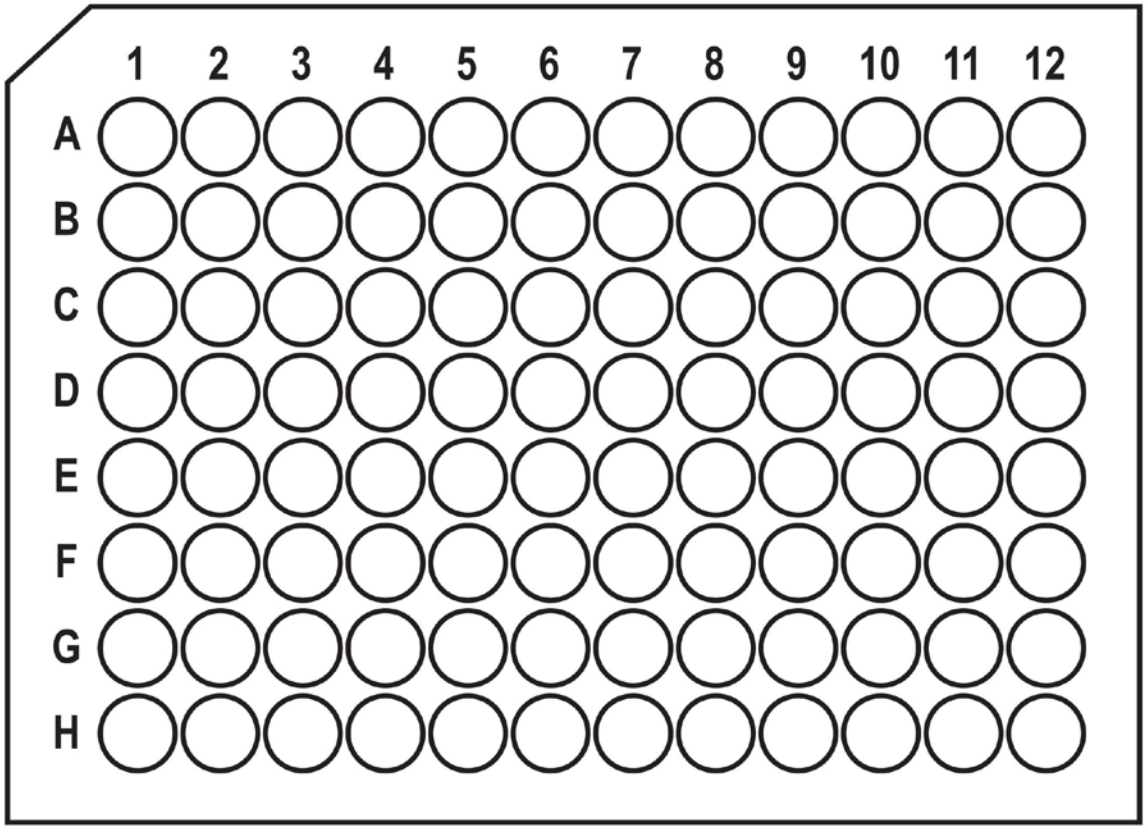
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