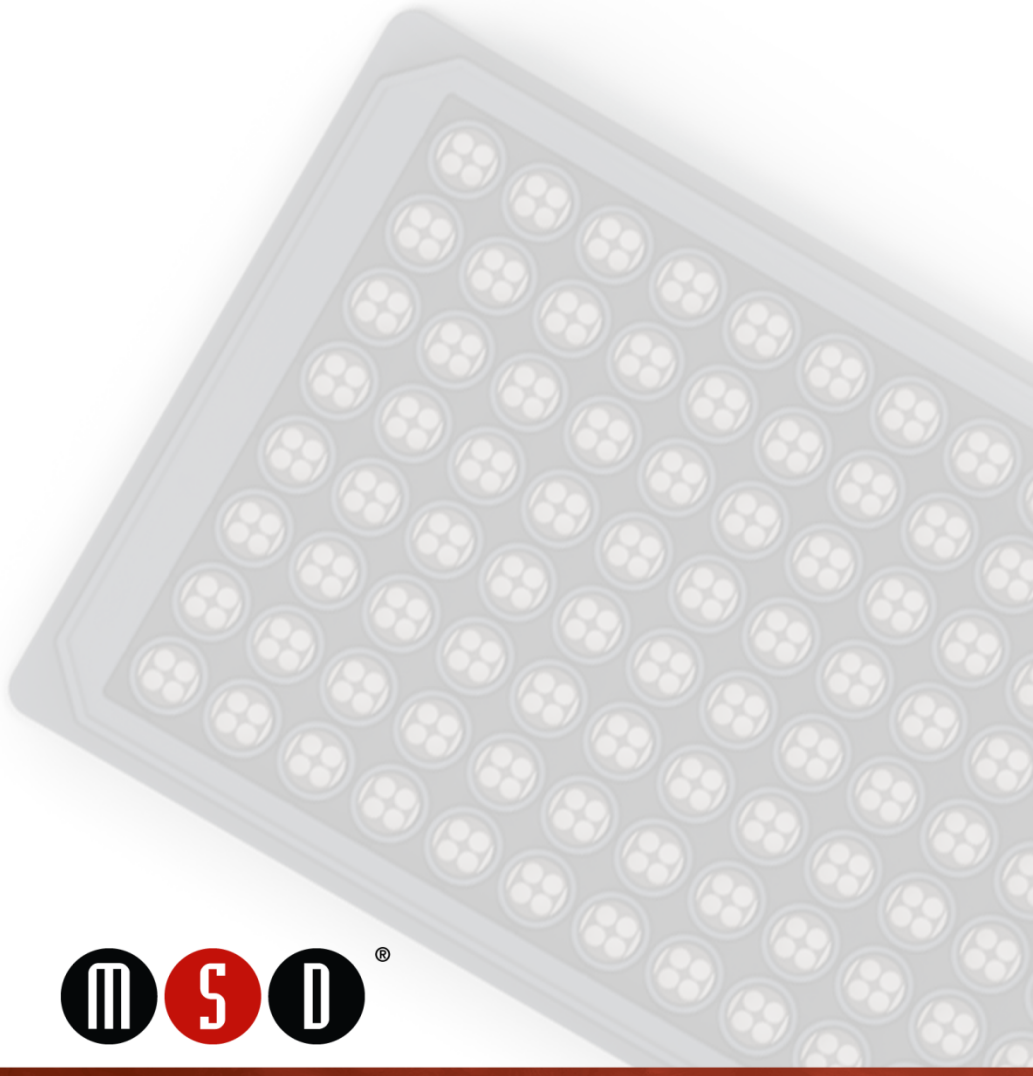
MSD® MULTI-SPOT Assay System

PSD-95 Kit

1-Plate Kit	K150QND-1
5-Plate Kit	K150QND-2
25-Plate Kit	K150QND-4



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MSD Cell Signaling Pathway Assays

PSD-95 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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Introduction

Postsynaptic density protein 95 (PSD-95), also known as SAP-90 (synapse-associated protein 90), belongs to a class of proteins referred to as MAGUKs (membrane-associated guanylate kinases).[1] Canonical MAGUK proteins contain one or three PDZ (PSD-95/discs large/zona occludens-1) domains, an SH3 domain, and a guanylate kinase homology domain. PSD-95 and several other MAGUK and PDZ containing proteins (e.g., PSD-93, SAP-97, SAP-102, MALS/Veli proteins) are enriched in the post synaptic density.[2,3] These proteins appear to regulate synaptic function by acting as molecular scaffolds for signaling machinery at synapses, in part by interaction of their PDZ domains with carboxy-terminal T/SXV motifs present on synaptic proteins including NMDA receptors and Shaker type K+ channels. PSD-95 has also been shown to link NMDA receptors to neuronal nitric oxide synthetase (nNOS), a downstream signal transduction protein, via a novel PDZ:PDZ interaction with nNOS.[4] PSD-95 interacts with the cytoplasmic tail of NMDA receptor subunits and shaker-type potassium channels, which is important for synaptic plasticity associated with NMDA receptor signaling. Overexpression or depletion of PSD-95 changes the ratio of excitatory to inhibitory synapses in hippocampal neurons. PSD-95 has been associated with autism, drug addiction, learning, and memory. Phosphorylation is critical for the regulation of PSD-95 function.[3]

Principle of the Assay

MSD phosphoprotein assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. PSD-95 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 an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

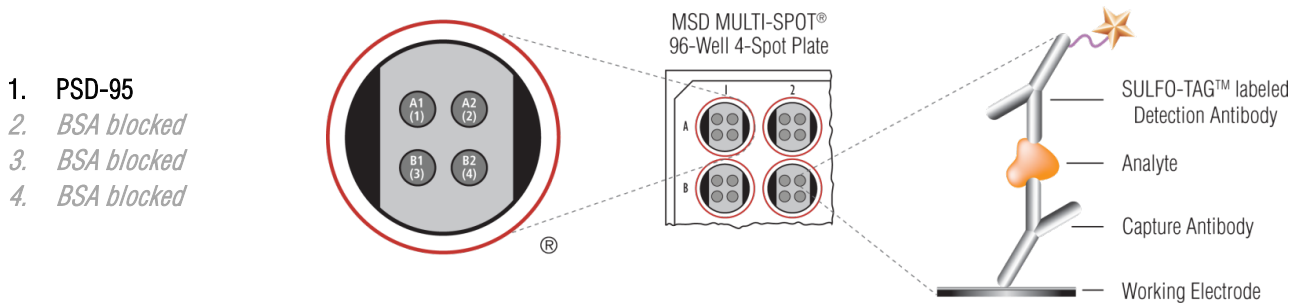


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K150QND-1	K150QND-2	K150QND-4
MULTI-SPOT® 96-Well, 4-Spot PSD-95 Plate N450QNA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-PSD-95 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Tris Lysis Buffer (1X) R60TX-3 (50 mL)	2–8°C	1 bottle	1 bottle	5 bottles
Tris Wash Buffer (10X) R61TX-2 (200 mL)	2–8°C	1 bottle	1 bottle	5 bottles
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 vials (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 A (dry powder) R93BA-4 (15 g)	RT	1 vial	1 vial	5 vials
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle	1 bottle	5 bottles

Additional Material and Equipment

- ❑ Appropriately sized tubes and bottles for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing serial dilutions
- ❑ 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 (rotary) capable of shaking at 500–1,000 rpm
- ❑ Deionized water

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 applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Best Practices

- Prepare samples in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- Do not shake the plate after adding the read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove the plate seals prior to reading the plate.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading and follow guidelines on how to read partial plates provided in the instrumental manual. Partially used plates may be stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Prepare Tris Wash Buffer

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

For one 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-A

For one plate, combine:

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

Prepare Complete Lysis Buffer

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

For one 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 Antibody Dilution Buffer

For one plate, combine:

- ❑ 1 mL of blocking solution-A
- ❑ 2 mL of 1X Tris Wash Buffer

Set aside on ice.
Store solutions containing Blocker A at 2–8°C for up to two weeks.

Prepare Detection Antibody Solution

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

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-PSD-95 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 one plate, combine:

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

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

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (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

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 >1 mM). 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.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Add Blocker

- ❑ Add 150 µL of blocking solution A to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (500–1,000 rpm) at room temperature.
- ❑ Prepare complete lysis buffer immediately prior to sample dilution.

STEP 2: Prepare Positive and Negative Cell Lysates

- ❑ Thaw cell lysate samples on ice and dilute them immediately before use in ice cold complete lysis buffer. Keep on ice during all manipulations and discard any unused thawed material.
- ❑ Lysate samples should be diluted to a working concentration of 1.2–800 µg/mL using complete lysis buffer as prepared above. This will provide 0.03–20 µg of lysate per well.

  Notes:

  - 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. You may prepare a dilution series at this point if desired.

STEP 3: Wash and Add Samples

- ❑ Wash the plate 3 times with 150–300 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 (500–1,000 rpm) at room temperature.

  Note: 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.

STEP 4: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 150–300 µL/well of Tris Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 5: Wash and Read

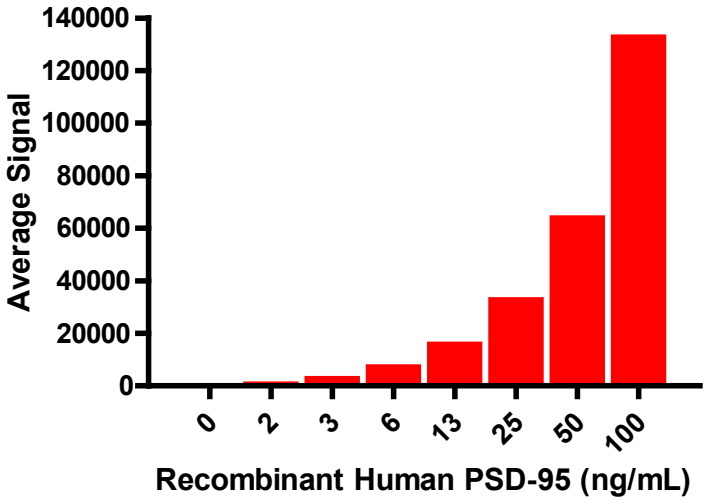
- ❑ Wash the plate 3 times with 150–300 µL/well of Tris Wash Buffer.
- ❑ Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on an MSD instrument. No incubation in read buffer is required.

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

Typical Data

Representative results for the PSD-95 Kit are illustrated below. The signal and ratio values provided are examples; individual results will vary depending upon the samples tested.

This assay was developed using recombinant human PSD-95 protein (data shown below) as well as with mouse brain tissue lysates (data not shown). Recombinant protein or mouse brain tissue lysates were added to MSD MULTI-SPOT 4-spot plates coated with anti-PSD-95 antibody on one of the four spatially distinct electrodes in each well. PSD-95 was detected with anti-PSD-95 antibody conjugated with SULFO-TAG.



Concentration (ng/mL)	PSD-95 Recombinant Protein		
	Average Signal	StdDev	%CV
0	71	7	9.9
1.6	2,726	146	5.3
3.1	4,853	111	2.3
6.3	9,269	171	1.8
13	17,959	277	1.5
25	34,839	1,024	2.9
50	66,028	1,032	1.6
100	134,827	1,485	1.1

Figure 2. Sample data generated with the PSD-95 Kit. Increased signal is observed with the titration of recombinant human PSD-95 protein.

Assay Components

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

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
PSD-95	Rabbit Monoclonal	Mouse Monoclonal	A

References

1. McGee AW and Bredt DS. Identification of an intramolecular interaction between the SH3 and guanylate kinase domains of PSD-95. J Biol Chem. 1999 Jun 18;274(25):17431-6.
2. Jo K, et al. Characterization of MALS/Velis-1, -2, and -3: a family of mammalian LIN-7 homologs enriched at brain synapses in association with the postsynaptic density-95/NMDA receptor postsynaptic complex. Genes Dev J Neurosci. 1999 Jun 1;19(11):4189-99.
3. Ziff EB. Enlightening the postsynaptic density. Neuron. 1997 Dec;19(6):1163-74.
4. Karen S, et al. PSD-95 Assembles a Ternary Complex with the N-Methyl-D-aspartic Acid Receptor and a Bivalent Neuronal NO Synthase PDZ Domain. J Biol Chem. 1999 Sep 24;274(39):27467-73.

Appendix: Suggested Cell Lysis Protocols

Preparation in Culture Flask or Petri Dish

Suspension Cells. Pellet cells by centrifugation at 500 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 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 once 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 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 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

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the PSD-95 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

- ❑ Add 150 µL/well of blocking solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 1 hour.

STEP 2: Prepare Samples

- ❑ Prepare complete lysis buffer just prior to sample dilution.
- ❑ Prepare positive and negative cell lysates and keep on ice until use.

STEP 3: Wash and Add Samples

- ❑ Wash plate 3 times with 150–300 µL/well of Tris Wash Buffer.
- ❑ Add 25 µL/well of sample (standards, controls, or unknowns).
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 3 hours.

STEP 4: Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 150–300 µL/well of Tris Wash Buffer.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 1 hour.

STEP 5: Wash and Read Plate

- ❑ Wash plate 3 times with 150–300 µL/well of Tris Wash Buffer.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument within 5 minutes of adding read buffer.

Plate Diagram