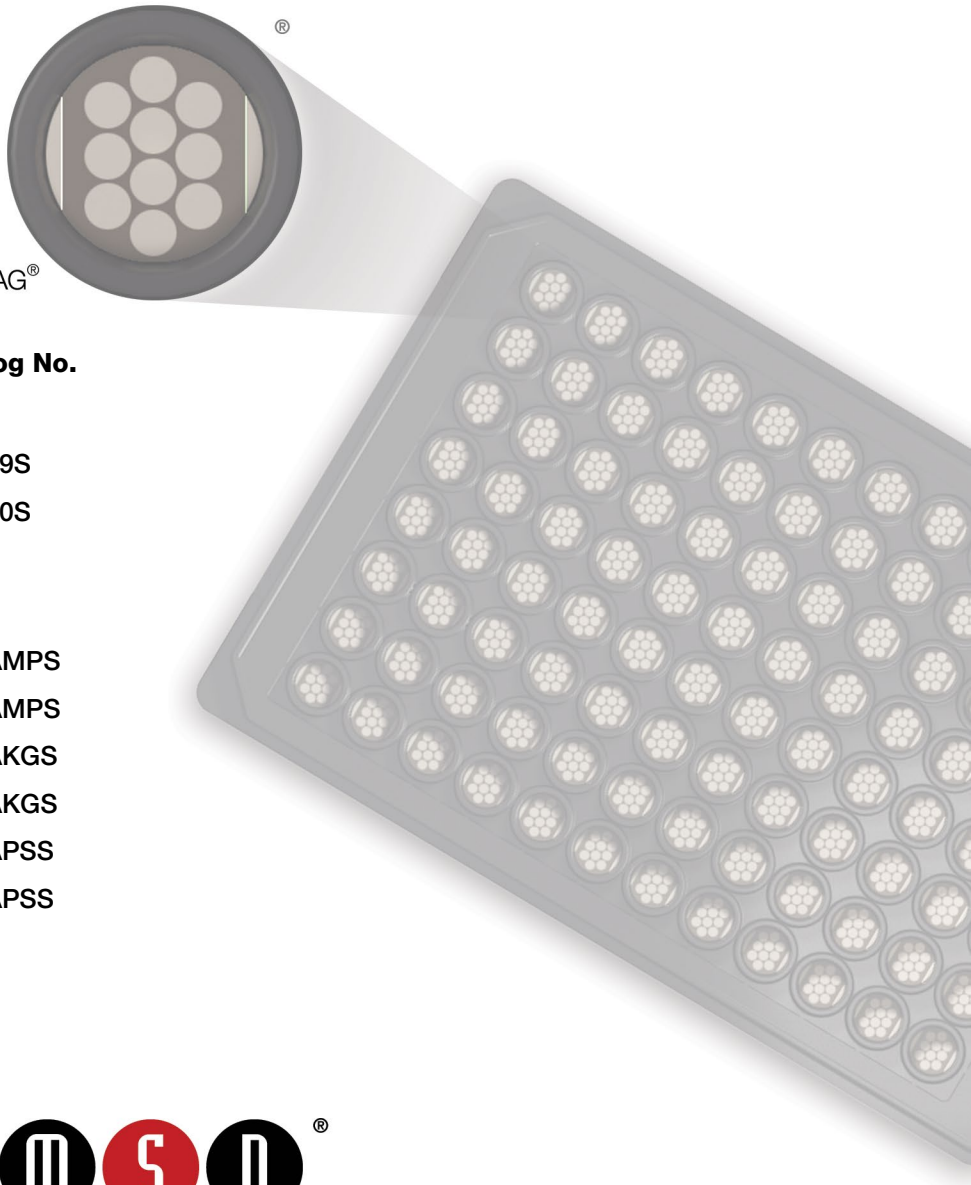
MSD® S-PLEX Platform

Neurology Panel 1 Kits

GFAP, Neurofilament L, Tau (total)

S-PLEX®
TrueSensitivity®
with TURBO-BOOST® & TURBO-TAG®



	Catalog No.
Multiplex Kits	
Neurology Panel 1 (human)	K15639S
Neurology Panel 1 (NHP)	K15640S
Singleplex Kits	
Human GFAP Kit	K151AMPS
NHP GFAP Kit	K156AMPS
Human Neurofilament L Kit	K151AKGS
NHP Neurofilament L Kit	K156AKGS
Human Tau (total) Kit	K151APSS
NHP Tau (total) Kit	K156APSS

MSD S-PLEX Platform

S-PLEX® Neurology Kits

S-PLEX GFAP Kits

S-PLEX Neurofilament L Kits

S-PLEX Tau (total) Kits

For use with human serum, EDTA plasma, citrate plasma, heparin plasma, cerebral spinal fluid (CSF), and cell culture supernatants, as well as NHP serum, EDTA plasma, and CSF.

Instrument Supported:

- SECTOR™ plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instruments

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®

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Introduction

The S-PLEX Neurology Panel 1 kits employ S-PLEX technology in a multiplex assay format. The kit measures three biomarkers that are important in neurological and neurodegenerative disorders. These are Glial Fibrillary Acidic Protein (**GFAP**), all forms of Tau (**Tau (total)**), and Neurofilament L (**NF-L**). S-PLEX Neurology Panel 1 kits are available to detect these biomarkers in multiple sample types across human and NHP species.

S-PLEX is MSD's ultrasensitive platform. It can dramatically improve the sensitivity of immunoassays, thus reducing the lower limit of detection (LLOD) by 10- to 1000-fold over other assay methods. Results vary from assay to assay, but detection limits in the low femtogram/mL range are common. These low detection limits enable the measurement of analytes at lower concentrations, reduce required sample volumes, and reduce the use of critical reagents.

S-PLEX uses electrochemiluminescence (ECL) technology, retaining its well-known advantages and superior analytical performance. The improved sensitivity of S-PLEX is due to the new TURBO-TAG® and TURBO-BOOST® reagents. When TURBO-TAG is combined with an antibody labeled with TURBO-BOOST, more ECL signal is generated than with other formats that use SULFO-TAG™ as the detection label. The S-PLEX platform uses the same robust MSD® instruments as other MSD assays. The protocol for S-PLEX is also straightforward, similar to other MSD assay methods. It is comprised of three simple steps: (1) Assemble the immunoassay, (2) Enhance with a TURBO-TAG label, and (3) Read on an MSD instrument.

Principle of the Assay

The assays in the S-PLEX Neurology Panel 1 are sandwich immunoassays. Biotinylated capture antibodies are coupled to linkers, which self-assemble onto unique spots on the S-PLEX Multiplex 96-Well SECTOR Plate. Analytes in the sample bind to the capture reagents; detection antibodies conjugated with the TURBO-BOOST label bind to the analytes to complete the sandwich immunoassay (Figure 1). Once the sandwich immunoassay is complete, the TURBO-TAG label detect solution is added to enhance TURBO-BOOST labeled detection antibody. The user adds an MSD read buffer that creates the 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, which is proportional to the amount of analyte present in the sample and provides a quantitative measure of each analyte. Multiplex S-PLEX Neurology Panel 1 and individual S-PLEX GFAP, Neurofilament L, and Tau (total) assays are provided on 10-spot MULTI-SPOT® plates.

1. GFAP
2. -
3. Neurofilament L
4. -
5. -
6. -
7. -
8. -
9. -
10. Tau (total)

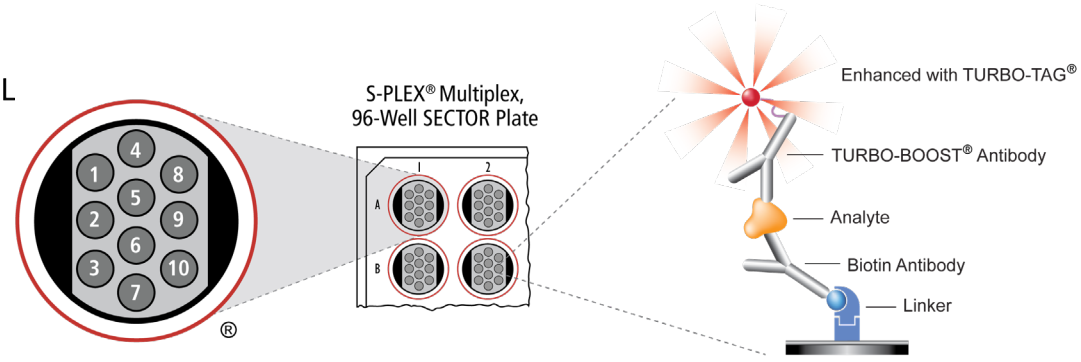


Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Kit Components

S-PLEX Neurology Panel 1 includes multiplex kits, individual GFAP, Neurofilament L, and Tau (total) assay kits, and custom kits with subsets of assays selected from the full panel. Kits have kit lot-specific (Table 1) and non-kit lot-specific reagents (Table 2, Table 3). Lot-specific information for each assay can be found in the certificate of analysis (COA).

Note: S-PLEX NHP Neurology kits share the same components as S-PLEX Human Neurology kits. S-PLEX GFAP, Neurofilament L, and Tau (total) singleplex kits also share the same components as the S-PLEX Neurology Panel 1, although they contain a single assay-specific TURBO-BOOST antibody.

See the **Catalog Numbers** section for complete kits.

Note: Components will be packaged by the indicated storage conditions for ease of storage and shipping.

Kit Lot-Specific Reagents and Components

Table 1. Reagents and components that are supplied with the S-PLEX Neurology kits

Reagent	Cap Color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
S-PLEX Neurology Panel 1 (human) Coating Solution	White	≤–70 °C	C2621-2	1.7 mL	1 vial	5 vial	25 vials	Blended biotinylated capture antibody coating solution
TURBO-BOOST Human GFAP Antibody*	Green	2–8 °C	D21AKF-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AKF-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human Neurofilament L Antibody**	Green	2–8 °C	D21AKG-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AKG-3	225 µL	–	1 vial	5 vials	
TURBO-BOOST Human Tau Antibody***	Green	2–8 °C	D21AKH-2	45 µL	1 vial	–	–	TURBO-BOOST conjugated detection antibody
			D21AKH-3	225 µL	–	1 vial	5 vials	
S-PLEX Neurology Panel 1 (human) Calibrator Blend	Blue	≤–70 °C	C01639-2	1 vial	1 vial	5 vials	25 vials	Liquid assay calibrator
Blocker S1 (100X)	Gray	≤–10 °C	R93AG-1	500 µL	1	1	5	Added to assay diluent. Reduces nonspecific signals.
Blocker S2 (100X)	Maroon	≤–10 °C	R93AH-1	500 µL	1	1	5	Added to assay diluent. Reduces nonspecific signals.
S-PLEX Enhance E1 (4X)	Yellow	≤–10 °C	R82AA-1	1.7 mL	1	5	25	Reagent 1 of 3 for Enhance Step
S-PLEX Enhance E2 (4X)	Orange	≤–10 °C	R82AB-1	1.7 mL	1	5	25	Reagent 2 of 3 for Enhance Step
S-PLEX Enhance E3 (200X)	Red	≤–70 °C	R82AC-1	50 µL	1	5	25	Reagent 3 of 3 for Enhance Step
S-PLEX Detect D1 (4X)	Purple	≤–70 °C	D20K0-2	1.7 mL	1	5	25	Reagent 1 of 2 for Detection Step (contains TURBO-TAG label)
S-PLEX Detect D2 (200X)	Blue	≤–70 °C	D20J0-2	50 µL	1	5	25	Reagent 2 of 2 for Detection Step

RT = room temperature
dash (—) = not applicable
* Individual GFAP assay kits (catalog numbers: K151AMPS and K156AMPS) include only TURBO-BOOST Human GFAP Antibody
** Individual Neurofilament L assay kits (catalog numbers: K151AKGS and K156AKGS) include only TURBO-BOOST Human Neurofilament L Antibody
*** Individual Tau (total) assay kits (catalog numbers: K151APSS and K156AKGS) include only TURBO-BOOST Human Tau (total) Antibody

Non-Kit Lot-Specific Reagents and Components

Table 2. Reagents and components that are supplied with the S-PLEX Neurology Panel 1 kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent used for preparing coating solution
Diluent 64	2–8 °C	R5DBB-1	18 mL	1 bottle	–	–	Assay and Antibody Diluent
		R5DBB-2	90 mL	–	1 bottle	5 bottles	
MSD GOLD™ Read Buffer B	RT	R60AM-1	18 mL	1 bottle	–	–	Buffer to catalyze the electrochemiluminescence reaction
		R60AM-2	90 mL	–	1 bottle	5 bottles	

RT = room temperature
dash (—) = not applicable

Table 3. Plates that are supplied with the S-PLEX Neurology Panel 1 kits and their instrument compatibility

Reagent	Storage	Catalog No.	Quantity Supplied			Instrument Compatibility	Description
			1 Plate	5 Plates	25 Plates		
S-PLEX Multiplex 96-Well SECTOR Plate	2–8 °C	N05396A-1	1 plate	5 plates	25 plates	MESO SECTOR S 600 MESO SECTOR S 600MM MESO QuickPlex SQ 120 MESO QuickPlex SQ 120MM	Plates for coating with capture antibodies

dash (—) = not applicable

Additional Materials and Equipment

Materials

- ❑ Adhesive plate seals
- ❑ Micropipettes with filtered tips
- ❑ Tubes (polypropylene microcentrifuge tubes, conical tubes, library tubes)
- ❑ Serological pipettes and pipette controller
- ❑ Reagent reservoir
- ❑ Plastic bottles
- ❑ Wet ice and ice bucket
- ❑ Deionized water
- ❑ Molecular biology grade water
- ❑ MSD Wash Buffer (catalog no. R61AA-1) used at 1X
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T)

Equipment

- ❑ Microtiter plate shaker capable of shaking at 500–1,000 rpm
- ❑ Microtiter plate shaker capable of shaking at 500–1,000 rpm and maintaining a controlled temperature of 27 °C (e.g., Kisker heated plate shaker)
- ❑ Plate-washing equipment (automated plate washer or multi-channel pipette)
- ❑ Vortex mixer
- ❑ Water bath
- ❑ Microcentrifuge

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay 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.

Best Practices

- Mixing or substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
- Bring frozen diluents, E1, E2, and D1 reagents to room temperature in a 22–25 °C water bath before use. If a controlled water bath is not available, thaw at room temperature. Ensure that diluents, E1, E2, and D1 reagents are fully thawed and equilibrated to room temperature before use. Mix well after thawing and before use.
- Thaw frozen vials of E3 and D2 reagents on ice until needed. Ensure that E3 and D2 reagents are fully thawed before use. Mix well after thawing and before use.
- To avoid cross-contamination between vials, open vials for each protocol step one at a time (vial caps are color-coded), use filtered pipette tips, and use a fresh pipette tip for each reagent addition.
- Prepare Calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid bubbles in wells during all pipetting steps, as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD Plate.
- Plate shaking should be vigorous, with a rotary motion between 500–1,000 rpm. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
- Use a new adhesive plate seal for each incubation step.
- When washing S-PLEX assays, the best results are obtained by using a low-dispense flow rate and by positioning dispenser tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This is most important after the detection solution incubation step. See **Appendix A** for more information on plate-washing recommendations.
- When performing manual plate washing using a multi-channel pipette, plates should be washed using at least 150 µL of wash buffer per well.
- Do not allow plates to dry after washing steps. Solutions associated with the next assay step should be added to the plate immediately after washing.
- Remove the plate seal before reading the plate.
- Make sure that the read buffer is at room temperature when adding it to the plate.
- Do not shake the plate after adding read buffer.
- To improve interplate precision, keep time intervals consistent between adding read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples, and repeat the assay.
- If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.
- Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solutions to light. Keep stocks of S-PLEX Detect D1 reagent in the dark. During the detection incubation step, plates do not need to be shielded from light except for direct sunlight.

Recommended Protocol

Bring all reagents to room temperature and refer to the **Best Practices** section (above) before beginning the protocol.

Reagents prepared at each step are sufficient for a one-plate experiment.

CRITICAL: Incubation temperatures can affect assay signals and sensitivity. For optimal results, follow the recommendations provided for each incubation step.

STEP 1: ASSEMBLE

Prepare Coating Solution

MSD provides the S-PLEX Neurology Panel 1 (human) Coating Solution as a 4X stock solution. Thaw the frozen vials and bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

Prepare the coating solution immediately before use by combining the following reagents. Vortex briefly to mix.

- ❑ 4,500 µL Diluent 100
- ❑ 1,500 µL of S-PLEX Neurology Panel 1 (human) Coating Solution ◯

Coat the Plate

- ❑ Wash the uncoated plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T. Prewashing the plate has been shown to increase signals and improve sensitivity in many assays.
- ❑ Add 50 µL of the coating solution to each well. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour.

Note: While the coated plate is incubating, prepare the blocking solution, calibrators, and diluted samples.

Prepare Blocking Solution

Blocking solution is the assay diluent supplemented with Blocker S1 and Blocker S2 and is designed to reduce nonspecific binding in the sample matrix. MSD provides Blocker S1 and Blocker S2 as 100X stock solutions. Vortex each vial to mix and spin down briefly before use.

Prepare the blocking solution by combining the following reagents. Vortex briefly to mix.

- ❑ 3,430 µL of Diluent 64
- ❑ 35 µL of 100X Blocker S1 ●
- ❑ 35 µL of 100X Blocker S2 ●

Notes:

- One vial each of Blocker S1 and Blocker S2 is sufficient for blocking 5 plates. If fewer than 5 plates are run, the unused Blocker S1 and Blocker S2 should be frozen immediately. The reagent is stable through 5 freeze-thaw cycles.
- The blocking solution should be added to the plate before sample addition.

Prepare Calibrator Dilutions

MSD supplies a multi analyte liquid calibrator that is 20-fold more concentrated than the recommended highest calibrator concentration (Standard 1). We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank (Figure 2). Thaw the stock calibrator and keep on ice, then add to Diluent 64 at room temperature to make the calibration curve solutions.

Note: Discard any unused, diluted calibration solutions. For the lot-specific concentration of the calibrator, refer to the COA supplied with the kit. You can also find the COA at www.mesoscale.com.

Prepare the standards plus a zero standard for up to 4 replicates (Figure 2):

- ❑ Prepare Standard 1 by adding 15 µL of stock calibrator to 285 µL of Diluent 64. Mix by vortexing.
- ❑ Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 64. Mix by vortexing.
- ❑ Repeat 4-fold serial dilutions five additional times to generate Standards 3–7. Mix by vortexing between each serial dilution.
- ❑ Use Diluent 64 as Standard 8 (zero standard).

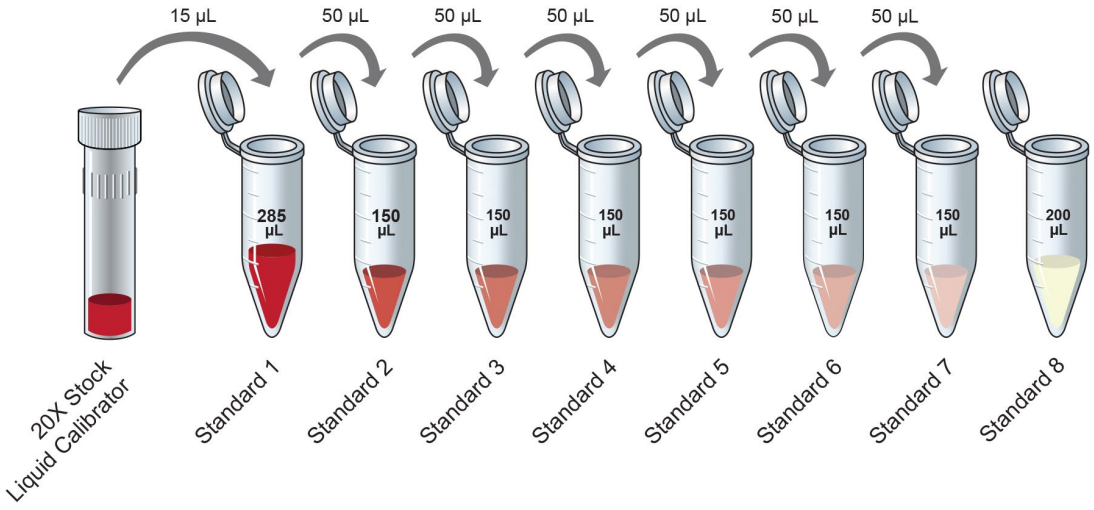


Figure 2. Dilution schema for preparation of Calibrator Standards

Sample Collection and Handling

General guidelines for sample collection, storage, and handling are presented below. If possible, use published guidelines.[1-5] Evaluate sample stability under the selected method as needed.

- **Serum and plasma**. When preparing serum, allow samples to clot for 2 hours at room temperature. If there are visible particulates, centrifuge for 20 minutes at 2,000 × *g* before using or freezing. Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge plasma for 20 minutes at 2,000 × *g* within 30 minutes of collection. Use immediately or freeze.
- **CSF**: MSD recommends reviewing current literature and protocols for the collection and handling of CSF samples or the use of published guidelines.[4]
- **Other samples**. Use immediately or freeze.

Freeze all samples in suitably sized aliquots; they may be stored at ≤–10 °C until needed. Repeated freeze-thaw of samples is not recommended. After thawing, centrifuge samples at 2,000 × *g* for 3 minutes to remove particulates before sample preparation. Hold on wet ice or at 2–8 °C until used in the assay.

Dilute Samples

For assays in the S-PLEX Neurology Panel 1, MSD recommends 2-fold dilutions for most human and NHP samples. Analyte levels in CSF samples can vary greatly and may benefit from 10- to 20-fold dilution. Dilute samples with Diluent 64. For example, to dilute samples 2-fold, add 30 µL of sample to 30 µL of Diluent 64. The assay requires 25 µL/well of the diluted sample. We recommend running at least two replicates per sample. You may conserve the sample by using a higher dilution. The dilution factor for other sample types will need to be optimized. The kit includes diluent sufficient for running samples in duplicates.

Add Calibrators and Sample

- ❑ After coating incubation completion, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
- ❑ Add 25 µL of calibrator or sample to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1.5 hours.

Note: CRITICAL: Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity. For best results, perform this incubation step between 22 °C and 27 °C.

Prepare TURBO-BOOST Antibody Solution

Multiplex Neurology Kits

MSD provides each TURBO-BOOST detection antibody separately as a 200X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use. Bring all reagents to room temperature. Vortex each vial to mix and spin down briefly before use.

Prepare the TURBO-BOOST antibody solution by combining the following reagents. Vortex briefly to mix.

- ❑ 5,910 µL of Diluent 64
- ❑ 30 µL of TURBO-BOOST Human GFAP Antibody
- ❑ 30 µL of TURBO-BOOST Human Neurofilament L Antibody
- ❑ 30 µL of TURBO-BOOST Human Tau Antibody

Individual GFAP, Neurofilament L, and Tau (total) Kits

For one plate, combine the following reagents. Vortex to mix.

- ❑ 5,970 µL of Diluent 64
- ❑ 30 µL of supplied TURBO-BOOST detection Antibody

Custom Multiplex Neurology kits

For one plate, combine 30 µL of each supplied TURBO-BOOST detection antibody with enough Diluent 64 to bring the final volume to 6,000 µL. Vortex briefly to mix.

Add TURBO-BOOST Antibody Solution

- ❑ After calibrator and sample incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of TURBO-BOOST antibody solution to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 1 hour.

Notes:

- CRITICAL: Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity. For best results, perform this incubation step between 22 °C and 27 °C.
- While the TURBO-BOOST antibody solution is incubating, thaw 1 vial each of S-PLEX Enhance E1 and E2 reagents at room temperature and E3 reagent on ice.

STEP 2: ENHANCE

Prepare Enhance Solution

Prepare enhance solution up to 30 minutes before use. Vortex each thawed vial to mix and spin down briefly before use.

Prepare enhance solution by combining the following reagents. Vortex briefly to mix.

- ❑ 2,970 µL Molecular Biology Grade Water
- ❑ 1,500 µL of 4X S-PLEX Enhance E1
- ❑ 1,500 µL of 4X S-PLEX Enhance E2
- ❑ 30 µL of 200X S-PLEX Enhance E3

Note: S-PLEX Enhance E3 stock solution is viscous. Pipette slowly to avoid bubble formation in the pipette tip and to ensure an accurate volume is pipetted.

Add Enhance Solution

- ❑ After TURBO-BOOST antibody incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of enhance solution to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at room temperature for 30 minutes.

Notes:

- CRITICAL: Incubation temperatures below 22 °C for this step can negatively affect assay signals and sensitivity. For best results, perform this incubation step between 22 °C and 27 °C
- While the enhance solution is incubating, thaw 1 vial each of S-PLEX Detect D1 at room temperature and Detect D2 on ice.
- CRITICAL: The TURBO-TAG detection incubation (next step) requires incubation at 27 °C. Upon completion of the enhance solution incubation, prepare a shaker at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

Prepare TURBO-TAG Detection Solution

Prepare the TURBO-TAG detection solution up to 30 minutes before use. Vortex each thawed vial to mix and spin down briefly before use.

Prepare TURBO-TAG detection solution by combining the following reagents. Vortex briefly to mix.

- ❑ 4,470 µL Molecular Biology Grade Water
- ❑ 1,500 µL of 4X S-PLEX Detect D1
- ❑ 30 µL of 200X S-PLEX Detect D2

Notes:

- CRITICAL: Avoid prolonged exposure of the S-PLEX Detect D1 reagent and the detection solution to light.
- S-PLEX Detect D2 solution is viscous. Pipette slowly to avoid bubble formation in the tip and to ensure an accurate volume is pipetted.

Add TURBO-TAG Detection Solution

- ❑ After enhance solution incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of TURBO-TAG detection solution to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate with shaking (~700 rpm) at 27 °C for 1 hour.

Note: CRITICAL: The incubation temperature for this step can affect the background and assay signals, thereby affecting the assay sensitivity. It is highly recommended that TURBO-TAG detection be performed at 27 °C. If you do not have access to a temperature-controlled shaker, a plate shaker can be placed inside an incubator maintaining 27 °C.

STEP 3: READ

- ❑ After TURBO-TAG detection incubation, wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T using a gentle wash step.

Notes:

- CRITICAL: For this final wash step, the best results are obtained by using a low-dispense flow rate and by positioning dispense tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the wall). See Appendix A for more information on plate washing recommendations if using an automated plate washer.
- Do not allow plates to dry after the final wash step. Proceed to add read buffer immediately after washing the plate.

Add Read Buffer

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

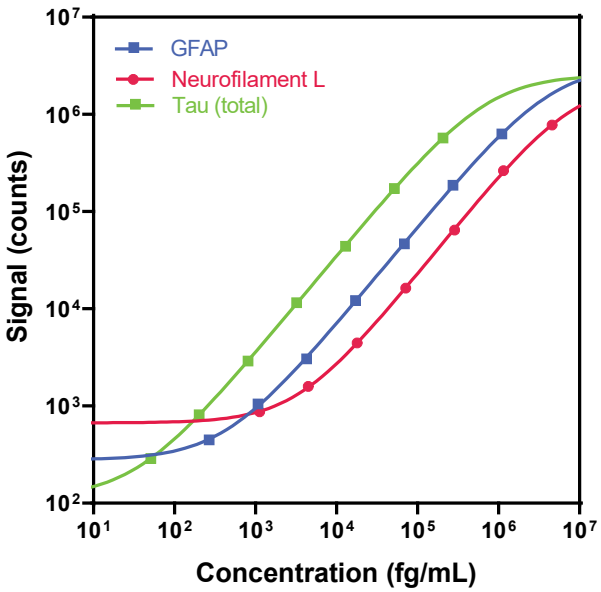
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well and read on an MSD reader. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

Note: CRITICAL: Refer to the plate-instrument compatibility table (Table 3) to ensure the correct plate is read on the compatible instrument. SECTOR plates are compatible with SECTOR and QuickPlex SQ120 instruments.

Assay Performance

A representative data set from the development studies for the S-PLEX Neurology Panel 1 kit is presented below. The kit release specifications for precision, accuracy, and sensitivity for each kit lot can be found in the lot-specific COA. The lot-specific COA is supplied with the kit and is available for download at www.mesoscale.com.

Representative Calibrator Curve



The calibration curves used to calculate analyte concentrations were established by fitting the signals from the calibrators using a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting (Figure 3). The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analyte concentrations are determined from the electrochemiluminescence signals by back fitting to the calibration curve. These assays have a wide dynamic range, which allows accurate measurement of samples without the need for multiple dilutions or repeated testing.

Figure 3. Typical calibration curves for the S-PLEX Neurology Panel 1 kits

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero standard). The median LLOD and range shown below (Table 4) were calculated from multiple runs (N=39 runs) using three kit lots. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) are verified on a lot basis using a range of sample concentrations prepared by diluting the calibrator blend. The ULOQ and LLOQ are defined as the highest and lowest concentration, respectively, at which the CV of the calculated concentration is <30% for ULOQ; <25% for LLOQ, and the recovery is within 70% to 130% of the known value for ULOQ, and 80% to 120% for LLOQ. The LLOQ and ULOQ values shown below (Table 4) were calculated from five runs using a single kit lot.

Table 4. Representative performance data for each analyte in the S-PLEX Neurology Panel 1 kits

Assay	Median LLOD (fg/mL)	LLOD range (fg/mL)	LLOQ (fg/mL)	ULOQ (fg/mL)
GFAP	150	55–800	320	850,000
Neurofilament L	930	350–2,400	5,400	3,600,000
Tau (total)	37	12–140	120	160,000

Tested Samples

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, CSF, and cell culture supernatant, as well as CSF samples from individuals with neurological disorders, were tested at dilution listed in the table below (Table 5). Similarly, normal NHP (*Rhesus macaque* and *Cynomolgus macaque*) serum, EDTA plasma, and CSF samples were also tested. The concentrations reported in Table 5 are adjusted for sample dilution. Medians are calculated from all tested samples. Percent detected is the percentage of samples tested with concentrations at or above the LLOD.

Table 5. *Samples tested in the S-PLEX Neurology Panel 1 kits*

Species	Sample Type	Fold-dilution	Statistics	GFAP	Neurofilament L	Tau (total)
Human	Serum (N=16)	2	Median (fg/mL)	24,000	57,000	2,200
			Range (fg/mL)	15,000–60,000	24,000–350,000	410–64,000
			% Detected	100	100	100
	EDTA Plasma (N=10)	2	Median (fg/mL)	22,000	45,000	13,000
			Range (fg/mL)	14,000–59,000	26,000–170,000	2,700–73,000
			% Detected	100	100	100
	Citrate Plasma (N=10)	2	Median (fg/mL)	22,000	40,000	12,000
			Range (fg/mL)	11,000–51,000	21,000–150,000	1,700–60,000
			% Detected	100	100	100
	Heparin Plasma (N=10)	2	Median (fg/mL)	22,000	48,000	7,400
			Range (fg/mL)	15,000–62,000	21,000–170,000	2,400–71,000
			% Detected	100	100	100
	CSF (N=26)	10	Median (fg/mL)	790,000	2,700,000	210,000
			Range (fg/mL)	11,000–5,600,000	ND–16,000,000	ND–490,000
			% Detected	100	96	73
	Diseased CSF (N=12)	100	Median (fg/mL)	2,200,000	3,100,000	230,000
			Range (fg/mL)	800,000–8,100,000	1,000,000–16,000,000	100,000–380,000
			% Detected	100	100	100
NHP	Serum (N=18)	2	Median (fg/mL)	3,700	87,000	240
			Range (fg/mL)	1,200–15,000	33,000–190,000	ND–870
			% Detected	100	100	61
	EDTA Plasma (N=18)	2	Median (fg/mL)	3,600	76,000	2,900
			Range (fg/mL)	1,300–10,000	28,000–210,000	98–25,000
			% Detected	100	100	100
	CSF (N=4)	10	Median (fg/mL)	750,000	2,400,000	6,100
			Range (fg/mL)	240,000–1,000,000	1,300,000–12,000,000	5,100–7,300
			% Detected	100	100	100
—	Cell Culture Supernatant (N=7)	2 or 100 or 400	Median (fg/mL)	4,600	18,000	55,000
			Range (fg/mL)	1,900–AS	8,300–AS	11,000–AS
			% Detected	100	100	100

dash (—) = not applicable
AS = above Standard 1; ND = non-detectable

Parallelism

Normal human serum, EDTA plasma, citrate plasma, and heparin plasma samples were diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Normal human CSF samples were diluted 10-fold, 20-fold, 40-fold, and 80-fold before testing. Normal NHP CSF samples were diluted 5-fold, 10-fold, 20-fold, and 40-fold before testing. NHP parallelism data for GFAP, Neurofilament L, and Tau (total) assays are reported only for the NHP CSF samples; levels of these analytes in other NHP matrices were below the limit of detection in diluted samples. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration, except for human and NHP CSF, which were normalized to the dilution-adjusted, 10-fold dilution concentration for human and 5-fold dilution concentration for NHP (Table 6).

$$\%\, recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 6. Analyte percent recovery at various fold dilutions in each sample type

			GFAP		Neurofilament L		Tau (total)	
Species	Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Human	Serum (N=4)	2	100	—	100	—	100	—
		4	112	105–119	113	110–120	116	101–135
		8	115	107–132	123	117–133	129	106–186
		16	112	105–129	124	116–132	136	98–210
	EDTA Plasma (N=4)	2	100	—	100	—	100	—
		4	108	103–115	108	103–113	115	99–144
		8	108	103–120	112	105–118	120	99–168
		16	105	98–120	119	109–127	123	85–192
	Citrate Plasma (N=4)	2	100	—	100	—	100	—
		4	107	102–116	108	106–110	116	101–131
		8	106	97–123	111	104–118	124	102–185
		16	99	86–121	117	110–127	129	90–213
	Heparin Plasma (N=4)	2	100	—	100	—	100	—
		4	111	105–116	110	102–115	124	96–154
		8	114	107–128	118	112–131	135	102–198
		16	109	105–118	112	104–120	135	88–231
	CSF (N=4)	10	100	—	100	—	100	—
		20	97	93–99	98	94–104	101	99–103
		40	93	90–101	97	91–107	101	98–105
		80	92	85–98	98	92–104	101	97–106
NHP	CSF (N=4)	5	100	—	100	—	100	—
		10	106	104–110	101	97–105	104	99–112
		20	105	101–111	101	96–104	105	100–111
		40	108	100–114	101	94–105	105	102–110

dash (—) = not applicable

Dilution Linearity

To assess linearity, normal human serum, EDTA plasma, heparin plasma, citrate plasma, and cell culture supernatant, as well as normal NHP serum and EDTA plasma samples, were spiked with recombinant calibrators and diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution was normalized to the dilution-adjusted 2-fold concentration (Table 7).

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} * 100$$

Table 7. Analyte percent recovery at various dilutions in each sample type

			GFAP		Neurofilament L		Tau (total)	
Species	Sample Type	Fold Dilution	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range
Human	Serum (N=4)	2	100	—	100	—	100	—
		4	106	97–111	110	105–121	105	104–110
		8	102	88–111	116	105–134	107	103–116
		16	112	99–126	120	108–152	110	104–124
	EDTA Plasma (N=4)	2	100	—	100	—	100	—
		4	101	95–106	107	101–115	104	100–107
		8	101	91–106	113	104–130	105	100–108
		16	107	96–119	119	107–140	104	98–109
	Citrate Plasma (N=4)	2	100	—	100	—	100	—
		4	105	99–118	107	101–113	105	102–107
		8	104	96–116	113	103–130	106	102–111
		16	111	102–124	116	106–134	104	98–111
	Heparin Plasma (N=4)	2	100	—	100	—	100	—
		4	105	104–106	112	107–123	107	100–112
		8	107	105–110	114	104–129	108	99–114
		16	116	112–123	113	90–139	109	92–119
NHP	Serum (N=4)	2	100	—	100	—	100	—
		4	108	101–119	109	107–112	110	105–115
		8	109	98–126	115	110–122	115	105–125
		16	116	98–142	116	115–116	118	107–128
	EDTA Plasma (N=4)	2	100	—	100	—	100	—
		4	103	93–126	109	99–115	103	101–107
		8	103	93–142	111	97–120	103	97–110
		16	106	88–101	113	101–119	101	98–107
—	Cell Culture Supernatant (N=2)	2	100	—	100	—	100	—
		4	108	107–108	100	96–104	114	109–118
		8	107	106–107	100	95–104	115	110–119
		16	111	111–111	103	98–107	111	107–114

dash (—) = not applicable

Spike Recovery

Spike recovery measurements of different sample types across the quantitative range of the assays were evaluated. Normal human (serum, EDTA plasma, citrate plasma, heparin plasma, and CSF) and normal NHP samples (serum and EDTA plasma), along with cell culture supernatant, were spiked with calibrators at three levels (high, mid, and low) then diluted 2-fold. Samples may require additional dilution with assay diluent to reduce matrix effects. The average % recovery for each sample type is reported along with the % recovery range (Table 8).

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} * 100$$

Table 8. Analyte percent recovery at various dilutions in each sample type

		GFAP		Neurofilament L		Tau (total)	
Species	Sample Type	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range	Average% Recovery	%Recovery Range
Human	Serum (N=4)	71	61–79	88	76–94	91	51–109
	EDTA Plasma (N=4)	78	62–90	88	81–92	91	56–111
	Citrate Plasma (N=4)	79	62–93	94	78–101	96	50–126
	Heparin Plasma (N=4)	74	55–104	88	75–96	90	46–109
	CSF (N=4)	86	78–99	89	81–97	75	58–85
NHP	Serum (N=4)	75	56-86	81	72-92	111	100-126
	EDTA Plasma (N=4)	72	55-81	80	75-86	127	114-148
—	Cell Culture Supernatant (N=2)	92	90–93	86	87–85	107	110–103

dash (—) = not applicable

Assay Components

Antibodies

The antibody source species are described in Table 9.

Table 9. Antibody source species

Analyte	Source Species		Assay Generation
	MSD Capture Antibody	MSD Detection Antibody	
GFAP	Mouse Monoclonal	Mouse Monoclonal	B
Neurofilament L	Mouse Monoclonal	Mouse Monoclonal	A
Tau (total)	Mouse Monoclonal	Mouse Monoclonal	A

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4. Schoonenboom NS, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implications for use in clinical practice. Clin Chem. 2005;51:189-95.
5. Girgrah N, et al. Purification and characterization of the P-80 glycoprotein from human brain. Biochem J. 1988;256:351-6.

Catalog Numbers

Table 10. Catalog numbers associated with the S-PLEX Neurology Panel 1 kits

Kit Name	SECTOR Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit
S-PLEX Neurology Panel 1 (human)	K15639S-1	K15639S-2	K15639S-4
S-PLEX Neurology Panel 1 (NHP)	K15640S-1	K15640S-2	K15640S-4
S-PLEX Human GFAP Kit	K151AMPS-1	K151AMPS-2	K151AMPS-4
S-PLEX NHP GFAP Kit	K156AMPS-1	K156AMPS-2	K156AMPS-4
S-PLEX Human Neurofilament L Kit	K151AKGS-1	K151AKGS-2	K151AKGS-4
S-PLEX NHP Neurofilament L Kit	K156AKGS-1	K156AKGS-2	K156AKGS-4
S-PLEX Human Tau (total) Kit	K151APSS-1	K151APSS-2	K151APSS-4
S-PLEX NHP Tau (total) Kit	K156APSS-1	K156APSS-2	K156APSS-4
S-PLEX Custom Human Neurology	K151ANV-1	K151ANV-2	K151ANV-4
S-PLEX Custom NHP Neurology	K156ANV-1	K156ANV-2	K156ANV-4

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX assays, the best results are obtained using a low dispense flow rate and positioning dispense tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). Ensure that the aspiration tips are positioned at the outer edge of the well (e.g. horizontal aspirate offset toward the right side of the well) and tips do not touch the plate bottom. This low-flow rate dispense program is recommended for washing after the detection step in S-PLEX assays; all other steps can use default wash programs. However, for convenience, plates can be washed using the low dispense flow rate program for all assay wash steps.

We recommend creating a new program for your automated plate washer with the optimal settings before starting your S-PLEX assay. Example settings for a typical (MSD-recommended) wash program and the S-PLEX program are shown below for a common plate washer (Biotek Model 405 LS) (Table 11).

Table 11. *Parameters for customized programs on the Biotek 405 LS microplate washer*

Wash Program Parameters	Typical Wash Program Settings	NEW Recommended S-PLEX Neurology Wash Program Settings
Plate type	96	96
CYCLES		
Wash cycles	3	3
ASPIRATION		
Aspirate Type	TOP	TOP
Travel Rate	1 (4.1% 1.0 mm/second)	1 (4.1% 1.0 mm/second)
Aspirate Delay	0500 milliseconds	0500 milliseconds
Aspirate X-Position	-35	49
Aspirate Y-Position	-35	00
Aspirate Height	22	24 (insure that aspiration tips do not touch well bottom)
Secondary Aspirate?	NO	NO
DISPENSE		
Dispense Rate	05	02
Dispense Volume	0300 µL/well	0300 µL/well
Vacuum Delay Volume	0300 µL/well	0010 µL/well
Dispense X-Position	00	-45
Dispense Y-Position	00	00
Dispense Height	120	120
OPTS		
PRE		
Wash Pre dispense?	NO	NO
Bottom Wash?	NO	NO
MIDCYC		
Wash Shake?	NO	NO
Wash Soak?	NO	NO
Home Carrier?	NO	NO
Between Cycle Pre Dispense?	NO	NO
POST		
Final Aspirate?	YES	YES
Aspirate Type	TOP	TOP
Travel Rate	3	1 (4.1% 1.0 mm/sec)
Final Aspirate Delay	0500 milliseconds	0500 milliseconds
Final Aspirate X-Position	-35 (1.600 mm)	49
Final Aspirate Y-Position	-35 (1.600 mm)	0
Final Aspirate Height	22	24 (insure that aspiration tips do not touch well bottom)
Secondary Aspirate?	YES	NO
Final Aspirate Secondary X-Position	35 (1.600 mm)	-
Final Aspirate Secondary Y-Position	35 (1.600 mm)	-
Final Aspirate Secondary Height	22	-

Appendix B: Frequently Asked Questions

- **Can I extend capture, sample, or detection antibody incubation times?**

  The best practice is to follow the S-PLEX protocol as outlined in the product insert. The plate coating step should not be extended overnight.

- **Can all plate incubation steps be performed at 27 °C?**

  Yes. In our study, no changes in sensitivity and minimal signal differences were observed when all incubations were conducted at 27 °C.

- **Can the recommended plate washer program be used throughout the entire protocol?**

  Yes. However, the recommended washing program is most important after the TURBO-TAG incubation step.

- **Is it possible to store any of the working solutions after the components are mixed? If so, for how long and at what temperatures?**

  All working solutions are stable at room temperature for 30 minutes. For longer periods, they should be stored on ice. They can be stored at 2–8 °C for up to 4 hours. Equilibrate each solution to room temperature 10–15 minutes before use.

- **When should I thaw my reagents?**

  Enhance Solution: Start thawing E1 and E2 at room temperature and E3 on ice 30 minutes after the start of TURBO-BOOST antibody incubation.

  TURBO-TAG Detection Solution: Start thawing D1 at room temperature and D2 on ice right after the start of the incubation of Enhance Solution.

- **Which reagents are recommended to be stored on ice? What stocks should be stored in the dark?**

  Reagents E3 and D2 are recommended to be stored on ice (they rapidly thaw completely on ice). D1 should be treated similarly to SULFO-TAG conjugated antibodies, and prolonged light exposures should be avoided.

- **For which assay steps is molecular-grade water essential? Must it be used to prepare wash buffer?**

  Wash buffer can be prepared using deionized water. Use molecular-grade water to prepare the enhance/detect reagents.

- **Can Milli-Q water be used instead of molecular-grade water in the enhance/detect steps?**

  We recommend molecular-grade water because of its known qualities and rigorous testing. If the Milli-Q water is known to be of high quality and not contaminated, Milli-Q water can be used.

- **What volume of wash buffer is needed during plate washing?**

  We recommend at least 150 µL of wash buffer per well for each washing step. However, if an automated plate washer is used adjust the volume as per the guidance in **Appendix A**.

Summary Protocol

Bring all reagents to room temperature and refer to the **Best Practices** section (above) before beginning the protocol.

STEP 1: ASSEMBLE

Coat Plate with Biotin Antibody

- ❑ Prewash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of coating solution containing biotinylated capture antibody to each well. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal.
- ❑ Incubate at room temperature with shaking (700 rpm) for 1 hour.

Add Samples and Calibrators

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
- ❑ Add 25 µL of calibrator or sample to each well. Seal the plate with an adhesive plate seal.
- ❑ Incubate at room temperature with shaking (700 rpm) for 1.5 hours. CRITICAL: For best results, perform this incubation step between 22 °C and 27 °C.

Add TURBO-BOOST Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of TURBO-BOOST antibody solution to each well. Seal the plate with an adhesive plate seal.
- ❑ Incubate at room temperature with shaking (700 rpm) for 1 hour. CRITICAL: For best results, perform this incubation step between 22 °C and 27 °C.

STEP 2: ENHANCE

Add Enhance Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of enhance solution to each well. Seal the plate with an adhesive plate seal.
- ❑ Incubate at room temperature with shaking (700 rpm) for 30 minutes. CRITICAL: For best results, perform this incubation step between 22 °C and 27 °C.

Add TURBO-TAG Detection Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of TURBO-TAG detection solution to each well. Seal the plate with an adhesive plate seal.
- ❑ Incubate at 27 °C in a temperature-controlled chamber with shaking (700 rpm) for 1 hour.

STEP 3: READ

Add Read Buffer

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer or PBS-T using a washer program with low dispense speed. See **Appendix A** for more details.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well. Read the plate on an MSD instrument. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

Plate Diagram

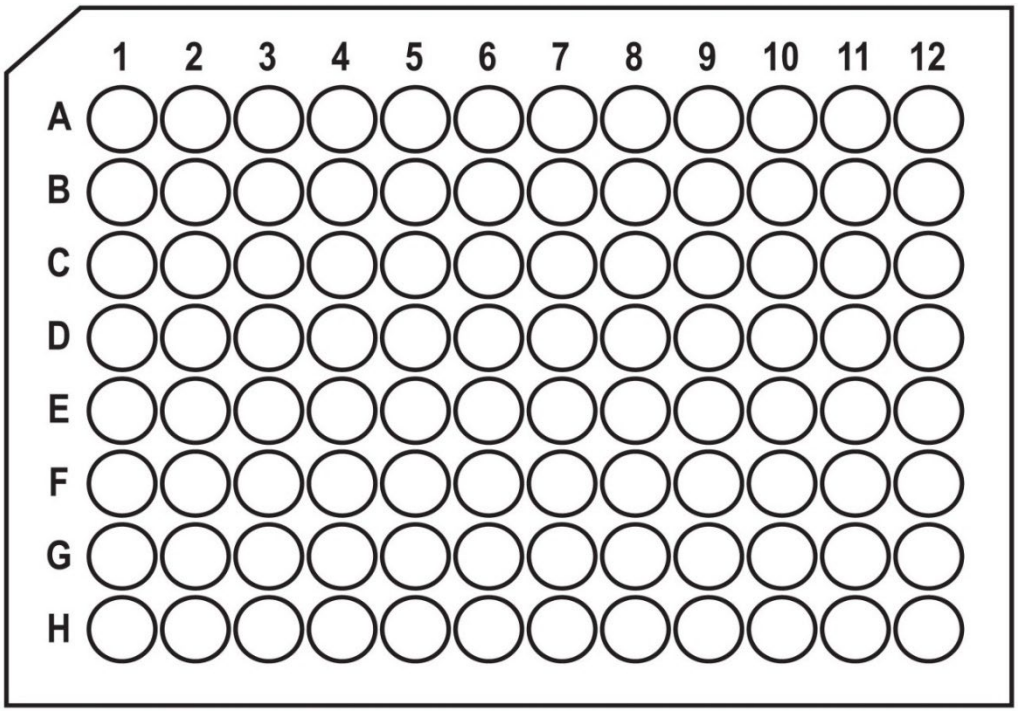

Figure 4. Plate Diagram.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

Figure 5. Sample plate layout that can be used for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.