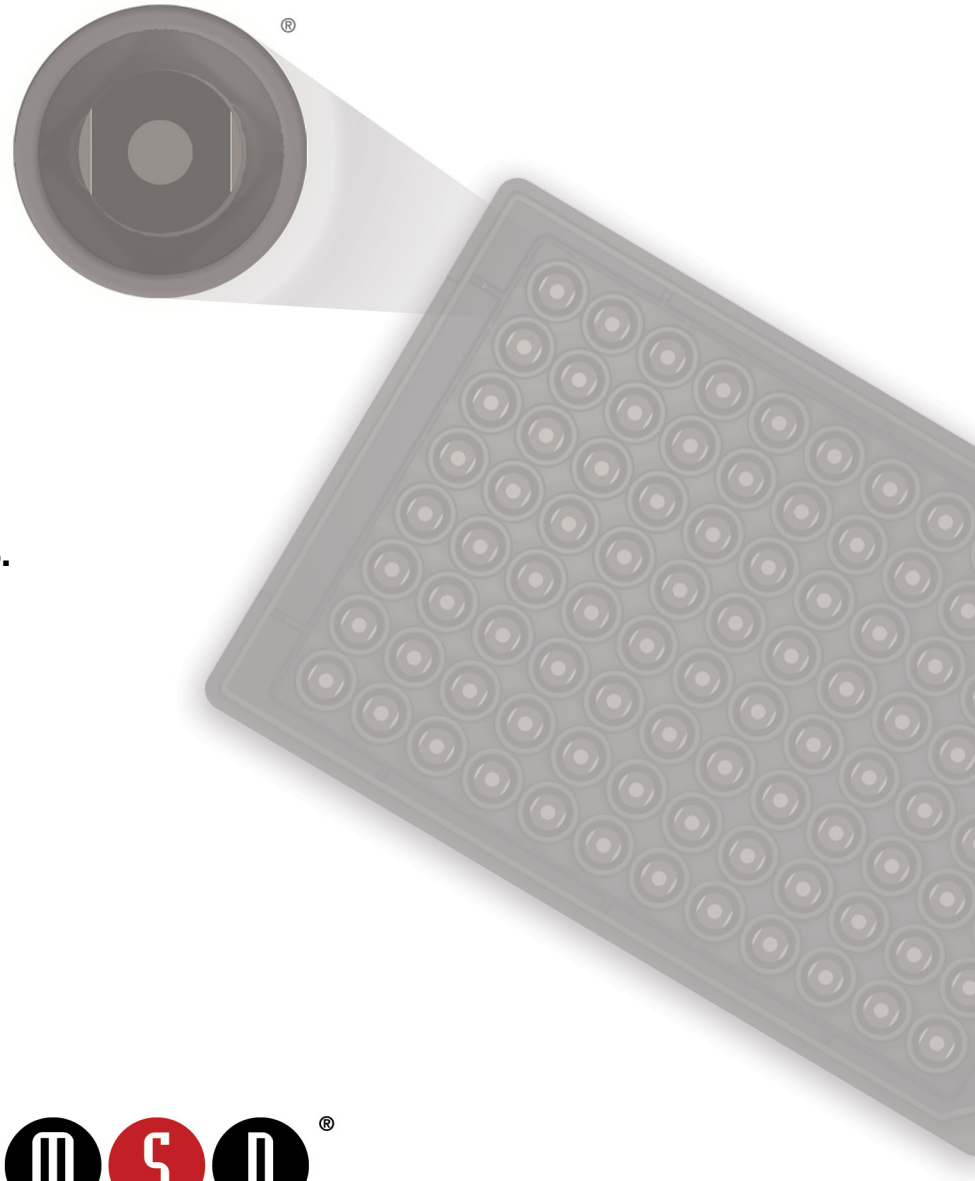
MSD® S-PLEX Platform

S-PLEX® Tau (pT231) Kit

S-PLEX® TrueSensitivity®



	Catalog No.
Human Tau (pT231) Kit	K151AGNS
NHP Tau (pT231) Kit	K156AGNS

MSD S-PLEX Platform

S-PLEX Human Tau (pT231) Kit

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

S-PLEX NHP Tau (pT231) Kit

For use with non-human primate (NHP) serum and EDTA plasma.

Instruments Supported:

- SECTOR™ plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instrument
- QuickPlex® plates for use on MESO QuickPlex Q 60MM instrument

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NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Table of Contents

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Introduction

The **S-PLEX Tau (pT231) Kit** is an ultrasensitive immunoassay. The assay measures phosphorylated Tau protein (Threonine231) in multiple sample types across human (serum, EDTA plasma, citrate plasma, heparin plasma, CSF, and cell culture supernatants) and NHP (serum and EDTA plasma) 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.

Principle of the Assay

S-PLEX assays use either S-PLEX 96-well SECTOR or QuickPlex plates (Figure 1) that are coated with streptavidin. These plates provide high sensitivity, consistent performance, and excellent inter- and intra-lot precision. S-PLEX Kits are supplied with a biotinylated capture antibody, a TURBO-BOOST conjugated detection antibody, a calibrator, assay and antibody diluents, and S-PLEX specific reagents.

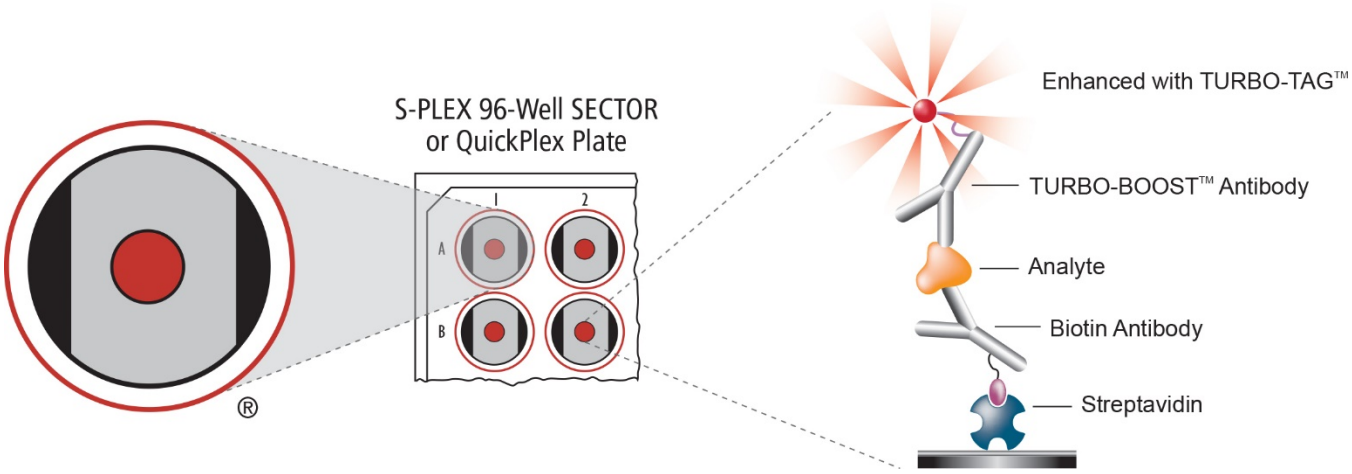

Figure 1. S-PLEX Singleplex Assay on an S-PLEX 96-well SECTOR or QuickPlex plate.

Performing an S-PLEX assay is similar to other MSD assays. The protocol is simple, robust, and uses common laboratory techniques. The protocol is depicted in Figure 2. The steps are outlined below.

ASSEMBLE

- ❑ Prepare coating solution containing biotin-conjugated capture antibody and S-PLEX Coating Reagent C1.
- ❑ Coat S-PLEX plate.
- ❑ Add samples and calibrators.
- ❑ Add TURBO-BOOST detection antibody.

ENHANCE

- ❑ Add S-PLEX enhance solution.
- ❑ Add S-PLEX detection solution. This detection solution includes the TURBO-TAG label that is required for the ECL signal. During this step, TURBO-TAG binds to the enhanced TURBO-BOOST. TURBO-BOOST or TURBO-TAG alone will not generate any signal.

READ

- ❑ Add MSD Read Buffer and read on an MSD instrument.

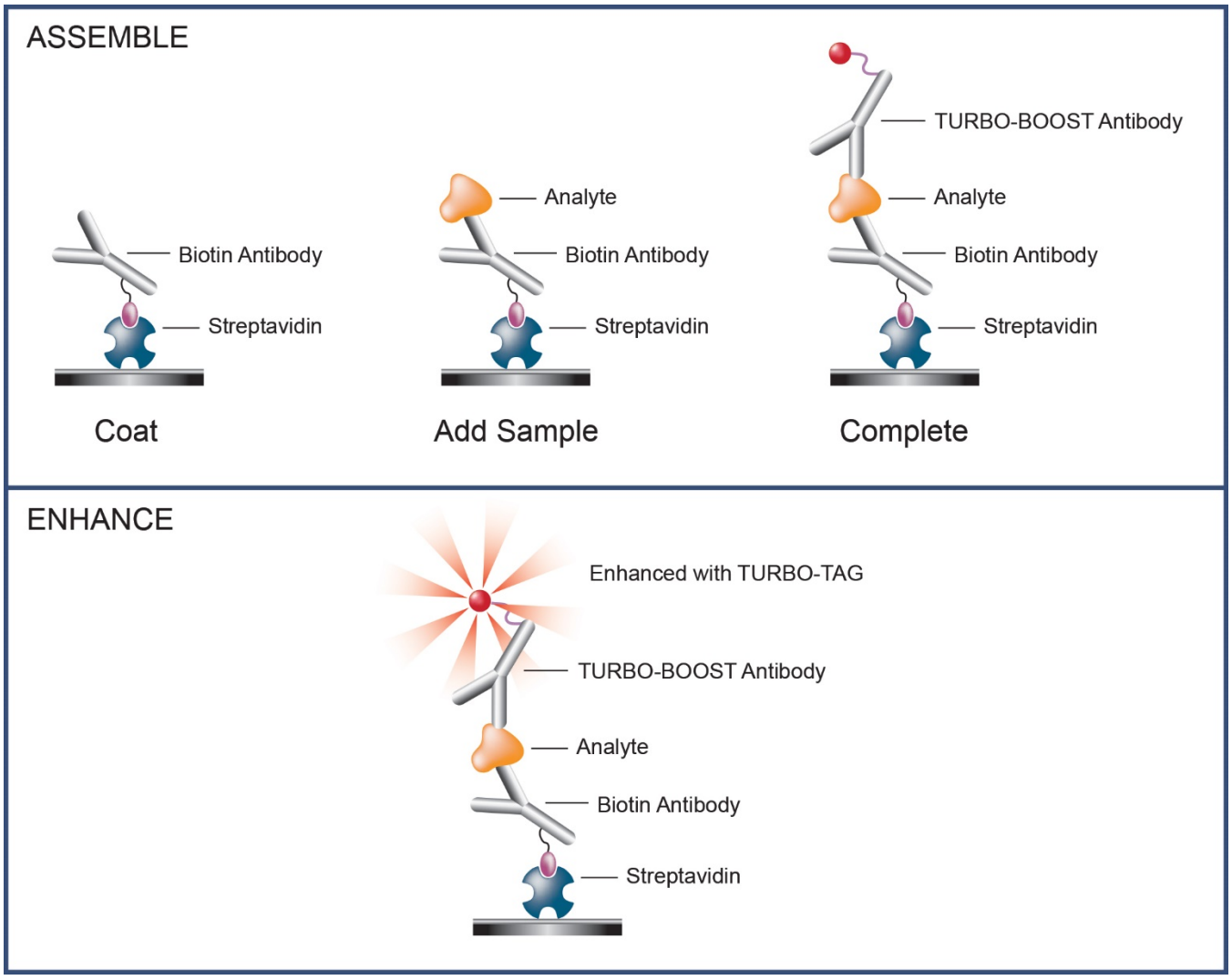

Figure 2. S-PLEX Assay format on an S-PLEX 96-well SECTOR or QuickPlex Plate.

Kit Components

S-PLEX assay kits are available as singleplex assays in 1-, 5-, and 25-plate sizes. S-PLEX assay kits include kit lot-specific (Table 1) and non-kit lot-specific reagents (Table 2). Assay kits are available in two plate formats compatible with either SECTOR or QuickPlex instruments (Table 3).

Note: S-PLEX NHP Tau (pT231) Kit shares the same components as S-PLEX Human Tau (pT231) Kit.

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

Note: Components are packaged by storage conditions for ease of storage and shipping.

Kit Lot-Specific Reagents and Components

Table 1. Kit lot-specific reagents and components that are supplied with the S-PLEX Tau (pT231) Kit

Reagent	Cap color	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Biotin Human Tau (pT231) Antibody‡	○	2–8 °C	C2AGN-2	170 µL	1	—	—	Assay-specific biotinylated capture antibody
			C2AGN-3	850 µL	—	1	5	
TURBO-BOOST Human Tau Antibody‡	●	2–8 °C	D2AGP-2	45 µL	1	—	—	TURBO-BOOST conjugated detection antibody
			D2AGP-3	225 µL	—	1	5	
Human Tau (pT231) Calibrator	—	2–8 °C	C0AGN-2	1vial	1 vial	5 vials	25 vials	Contains analyte of known concentration, used for creating the standard curve for each assay.
S-PLEX Coating Reagent C1 (200X)	●	≤–70 °C	C20H0-3	300 µL	1	1	5	Reagent mixed with capture antibody for plate coating, enhances assay signals.
Blocker S1 (100X)	●	≤–10 °C	R93AG-1	500 µL	1	1	5	Added to assay diluent, reduces nonspecific signals.
S-PLEX Enhance E1 (4X)	●	≤–10 °C	R82AA-1	1.7 mL	1	5	25	Reagent 1 of 3 for Enhance Step
S-PLEX Enhance E2 (4X)	●	≤–10 °C	R82AB-1	1.7 mL	1	5	25	Reagent 2 of 3 for Enhance Step
S-PLEX Enhance E3 (200X)	●	≤–70 °C	R82AC-1	50 µL	1	5	25	Reagent 3 of 3 for Enhance Step
S-PLEX Detect D1 (4X)	●	≤–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)	●	≤–70 °C	D20J0-2	50 µL	1	5	25	Reagent 2 of 2 for detection step
Diluent 2	—	≤–10 °C	R51BB-4	8 mL	1 bottle	—	—	Assay diluent for samples and calibrator
			R51BB-3	40 mL	—	1 bottle	5 bottles	

Lot-specific information for each assay can be found in the certificate of analysis (COA).
Dash (—) = not applicable.
‡ = Biotin and TURBO-BOOST antibodies are shipped as an Antibody Set (Catalog Nos. B2AGN-2 for 1-plate and B2AGN-3 for 5- and 25-plate sizes).

Non-Kit Lot-Specific Reagents and Components

Table 2. Non-kit lot-specific reagents that are supplied with the S-PLEX Tau (pT231) Kit

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	Coating buffer for capture antibody and S-PLEX Coating Reagent C1
Diluent 59	2–8 °C	R50CB-2	8 mL	1 bottle	—	—	Antibody diluent for diluting the TURBO-BOOST antibody
		R50CB-4	40 mL	—	1 bottle	5 bottles	
MSD GOLD™ Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent 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 Kit and their instrument compatibility

Reagent	Storage	Catalog No.	Quantity Supplied			Instrument Compatibility	Description
			1 Plate	5 Plates	25 Plates		
S-PLEX 96-Well SECTOR Plate	2–8 °C	L45KA-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
S-PLEX 96-Well QuickPlex Plate	2–8 °C	L4BNA-1	1 plate	5 plates	25 plates	MESO QuickPlex Q 60MM	

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) diluted to 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., a Kisker heated plate shaker)
- Plate-washing equipment (automated plate washer or multichannel 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), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

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 one protocol step at a time (vial caps are color-coded). Close the cap after use. 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 on 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 all incubation steps.
- 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 multichannel pipette, plates should be washed using at least 150 µL of wash buffer per well. Excess residual volume after washing should be removed by gently tapping the plate on a paper towel.
- 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 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.

Important: Upon the first thaw, aliquot Diluent 2 into suitable volumes before refreezing.

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

A sample plate layout is shown in Figure 6 (last page).

STEP 1: ASSEMBLE

Prepare Coating Solution

Biotinylated capture antibody is provided as a 40X stock solution and S-PLEX Coating Reagent C1 as a 200X 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.

- ❑ 5,820 µL Diluent 100
- ❑ 150 µL of Biotin Human Tau (pT231) Antibody ○
- ❑ 30 µL of 200X S-PLEX Coating Reagent C1 ●

Notes:

- **CRITICAL:** Failure to add S-PLEX Coating Reagent C1 in the coating solution will drastically reduce the assay signal.
- The unused S-PLEX Coating Reagent C1 should be frozen immediately after use. The reagent is stable through 5 freeze-thaw cycles.

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 or overnight at 2–8 °C. Shaking is not required for the overnight coating incubation step.

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 is designed to reduce nonspecific binding in the sample matrix. Blocker S1 is provided as a 100X 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 blocking solution by combining the following reagents. Vortex briefly to mix.

- ❑ 3,465 µL of Diluent 2
- ❑ 35 µL of 100X Blocker S1 ◯

Notes:

- One vial of Blocker S1 is sufficient for blocking 5 plates. If fewer than 5 plates are run, the unused Blocker S1 should be frozen immediately after use. 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 lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1,000 µL of Diluent 2.

Prepare the highest calibrator concentration (Standard 1):

- ❑ Reconstitute lyophilized Tau (pT231) Calibrator by adding 1,000 µL of Diluent 2 to the vial. Invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15–30 minutes, and then vortex briefly using short pulses.

Note: Reconstituted calibrator is not stable when stored at 2–8 °C; however, it may be stored in aliquots at ≤–70 °C and is stable for one freeze-thaw cycle. 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 remaining standards plus a zero standard for up to 4 replicates (Figure 3):

- ❑ Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 2. 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 2 as Standard 8 (zero standard).

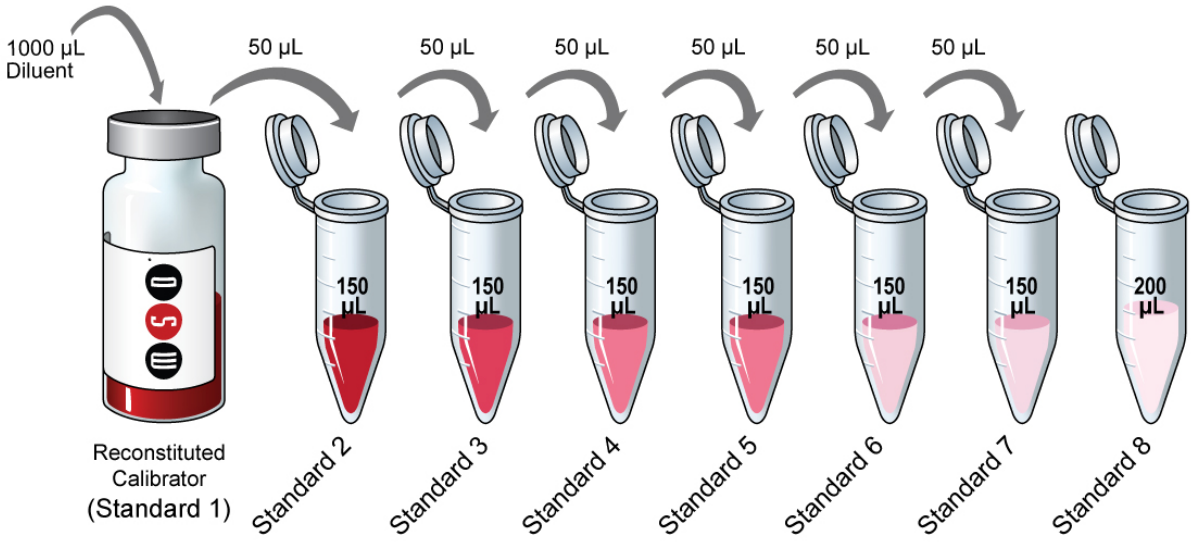

Figure 3. Dilution schema for preparation of calibrator standards.

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 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 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

Human serum, plasma, CSF, as well as NHP serum and plasma do not require dilution for measuring Tau (pT231). The assay requires 25 µL/well of sample. You may conserve the sample by using a higher dilution. The dilution factor for other sample types will need to be optimized. Additional diluent can be purchased at www.mesoscale.com.

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.

Prepare TURBO-BOOST Antibody Solution

TURBO-BOOST detection antibody is provided as a 200X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use. Bring all reagents to room temperature. Spin down the vial before use.

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

- ❑ 5,970 µL of Diluent 59
- ❑ 30 µL of TURBO-BOOST Human Tau Antibody 

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.

Note: 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 accurate pipetting volume.

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:

- 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 detection solution to light.
- S-PLEX Detect D2 solution is viscous. Pipette slowly to avoid bubble formation in the tip and to ensure accurate pipetting volume.

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

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

Add Read Buffer

- ❑ 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.

Note: 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.

- ❑ 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 SQ instruments. QuickPlex plates are ONLY compatible with the QuickPlex Q 60MM instrument.

Assay Performance

A representative data set for the S-PLEX Tau (pT231) assay is presented below (Figure 4; Table 4). The data represent the performance of the assay tested in a singleplex format. The data were generated during the development of the assay using a single kit lot. 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 and Sensitivity

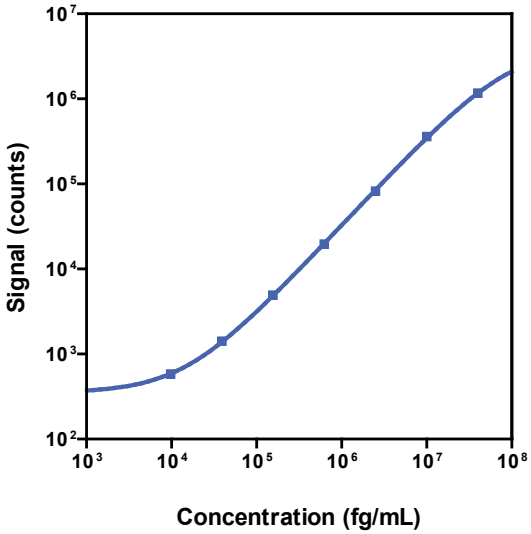

Figure 4. Typical calibrator curve for the S-PLEX Tau (pT231) Kit.

Table 4. LLOD, and LLOQ for the S-PLEX Tau (pT231) Kit

Suggested Sample Dilution	neat
LLOD (fg/mL)	3,100 range: 630–5,400
LLOQ (fg/mL)	15,000
ULOQ (fg/mL)	15,000,000

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. Analyte concentrations were determined from the ECL signals by back-fitting to the calibration curve.

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero Standard). Limits of quantification (LOQ) were first estimated based on the calibrator performance over multiple runs. The LLOQ (lower limit of quantification) and ULOQ (upper limit of quantification) were verified on a lot basis using a range of sample concentrations prepared by diluting the calibrator blend and assessing the accuracy (70% to 130% for ULOQ and 80% to 120% for LLOQ) and precision (30% for ULOQ and 20% for LLOQ) of the LOQ samples.

Tested Samples

Human Samples

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, CSF, and cell culture supernatant, as well as serum and CSF samples from individuals with neurological disorders were tested without dilution (Table 5). 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 Human Tau (pT231) Kit

Statistics	Serum (N = 21)	EDTA Plasma (N = 14)	Citrate Plasma (N = 14)	Heparin Plasma (N = 14)	CSF (N = 26)	Cell Culture Supernatants (N = 9)
Median (fg/mL)	ND	ND	ND	ND	ND	ND
Range (fg/mL)	ND–3,300	ND–3,400	ND–1,600	NA	ND–3,400	ND–27,000
% Detected	4	14	7	0	24	33

NA = not applicable.
ND = non-detectable (<LLOD).

Table 5. (continued)

Statistics	Diseased Serum (N = 6)	Diseased CSF (N = 3)
Median (fg/mL)	ND	2,500
Range (fg/mL)	NA	1,700–21,000
% Detected	0	100

NA = not applicable.
ND = non-detectable (<LLOD).

Non-Human Primate Samples

Normal NHP serum and EDTA plasma samples were tested without dilution (Table 6). Medians are calculated from all tested samples. Percent detected is the percentage of samples tested with concentrations at or above the LLOD.

Table 6. Samples tested in the S-PLEX NHP Tau (pT231) Kit

Species	Statistics	Serum (N = 5)	EDTA Plasma (N = 5)
Rhesus macaque	Median (fg/mL)	ND	ND
	Range (fg/mL)	NA	NA
	% Detected	0	0
Cynomolgus macaque	Median (fg/mL)	ND	ND
	Range (fg/mL)	NA	ND–2,400
	% Detected	0	20

NA = not applicable.
ND = non-detectable (<LLOD).

Dilution Linearity (Human)

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, CSF, and cell culture media samples were spiked with calibrator and tested at different dilutions (Table 7). Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

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

Fold Dilution	Serum		EDTA Plasma		Citrate Plasma		Heparin Plasma		CSF	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
neat	100	—	100	—	100	—	100	—	100	—
2	134	106–171	113	105–126	106	97–125	128	111–147	179	131–226
4	131	100–171	104	91–117	98	84–117	127	105–146	181	133–229
8	133	101–173	100	84–118	94	81–116	130	107–148	179	132–226

Dash (—) = not applicable.

Table 7. (continued)

Fold Dilution	RPMI	DMEM
	Average % Recovery	Average % Recovery
neat	111	377
2	107	363
4	112	368
8	111	377

Dilution Linearity (NHP)

Normal NHP serum and EDTA plasma samples were spiked with calibrator and tested at different dilutions (Table 8). Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

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

Species	Fold Dilution	Serum	EDTA Plasma
		Average % Recovery	Average % Recovery
Rhesus macaque	neat	100	100
	2	199	121
	4	219	121
	8	236	120
Cynomolgus macaque	neat	100	100
	2	227	128
	4	306	148
	8	373	156

Spike Recovery (Human)

Normal human serum, EDTA plasma, citrate plasma, heparin plasma, and cell culture media samples were spiked with calibrator at 3 levels (Table 9). Spiked samples were tested without dilution. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

Table 9. Spike and recovery measurement of different sample types at three spiked levels

Spike Level	Serum		EDTA Plasma		Citrate Plasma		Heparin Plasma		CSF	
	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
High	61	50–68	129	103–148	62	56–65	122	103–135	85	82–88
Mid	60	51–66	140	111–163	64	61–69	123	103–135	80	75–86
Low	60	50–69	129	109–142	63	51–69	115	92–125	83	82–84

Table 9. (continued)

Spike Level	RPMI	DMEM
	Average % Recovery	Average % Recovery
High	81	42
Mid	83	48
Low	84	46

Spike Recovery (NHP)

Normal NHP serum and EDTA plasma samples were spiked with calibrator at 3 levels (Table 10). Spiked samples were tested without dilution. Samples may require additional dilution with assay diluent to reduce matrix effects.

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

Table 10. Spike and recovery measurement of different sample types at three spiked levels

Species	Spike Level	Serum	EDTA Plasma
		Average % Recovery	Average % Recovery
Rhesus macaque	High	52	18
	Mid	50	19
	Low	54	25
Cynomolgus macaque	High	89	87
	Mid	85	78
	Low	82	84

Specificity

To assess specificity, the S-PLEX Human Tau (pT231) assay was tested against a larger panel of human analytes for nonspecific binding (Aβ42, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, FLT3L, G-CSF, G-FAP, GM-CSF, GRO-α, I-309, IFN-α2a, IFN-β, IFN-γ, IL-10, IL-12/23p40, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-17E/IL25, IL-17F, IL-18, IL-1RA, IL-1α, IL-1β, IL-2, IL-21, IL-22, IL-23, IL-27, IL-29, IL-2RA, IL-3, IL-31, IL-33, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, LRRK, LRRK2 (pS935), MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3α, MIP-5, NF-L, TARC, Tau (non-phosphorylated), TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, YKL-40).

Nonspecific binding was less than 0.5%.

$$\%\, nonspecificity\ = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

Species Cross-Reactivity

S-PLEX Human Tau (pT231) assay cross-reacts with non-human primate samples. S-PLEX NHP Tau (pT231) shares the same components as the S-PLEX Human Tau (pT231) Kit.

Assay Components

Calibrators

Full-length recombinant phosphorylated Tau (isoform tau441) protein expressed in a human cell line is used as a calibrator for the S-PLEX Tau (pT231) Kit.

Antibodies

The antibody source species are described in Table 11.

Table 11. Antibody source species

Analyte	Capture Antibody	Detection Antibody	Assay Generation
Tau (pT231)	Mouse Monoclonal	Mouse Monoclonal	A

References

1. Bowen RA, et al. Impact of blood collection devices on clinical chemistry assays. Clin Biochem. 2010;43:4-25.
2. Zhou H, et al. Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. Kidney. 2006;69:1471-6.
3. Thomas CE, et al. Urine collection and processing for protein biomarker discovery and quantification. Cancer Epidemiol Biomarkers & Prevention. 2010;19:953-9.
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.

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX Assays, 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 well). 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 S-PLEX 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 12). The only differences from typical wash program settings are the Dispense Rate and Dispense X-Position.

Table 12. Parameters for customized programs on the Biotek 405 LS microplate washer

Wash Program Parameters	Typical Wash Program Settings	Recommended S-PLEX 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 (1.600 mm)	-35 (1.600 mm)
Aspirate Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Aspirate Height	22	22
Secondary Aspirate?	NO	NO
DISPENSE		
Dispense Rate	05	02
Dispense Volume	0300 µL/well	0300 µL/well
Vacuum Delay Volume	0300 µL/well	0300 µL/well
Dispense X-Position	00 (0.000 mm)	-35 (1.600 mm)
Dispense Y-Position	00 (0.000 mm)	00 (0.000 mm)
Dispense Height	120 (15.245 mm)	120 (15.245 mm)
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	3
Final Aspirate Delay	0500 milliseconds	0500 milliseconds
Final Aspirate X-Position	-35 (1.600 mm)	-35 (1.600 mm)
Final Aspirate Y-Position	-35 (1.600 mm)	-35 (1.600 mm)
Final Aspirate Height	22	22
Secondary Aspirate?	YES	YES
Final Aspirate Secondary X-Position	35 (1.600 mm)	35 (1.600 mm)
Final Aspirate Secondary Y-Position	35 (1.600 mm)	35 (1.600 mm)
Final Aspirate Secondary Height	22	22

Appendix B: Frequently Asked Questions

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

  The best practice is to follow the S-PLEX protocol as outlined in the product insert. The plate coating step can be extended overnight, however. Once coating solution is added, store the plate overnight at 2–8 ºC without shaking. Equilibrate the plate to room temperature before proceeding with the next step.

- **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

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 and Coating Reagent C1 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, or overnight without shaking at 2–8 °C.

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.

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.

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.

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 shaker 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.

Catalog Numbers

Table 13. Catalog numbers associated with the S-PLEX Tau (pT231) Kit

Kit Name	SECTOR Plate			QuickPlex Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit	1-Plate Kit	5-Plate Kit	25-Plate Kit
S-PLEX Human Tau (pT231)	K151AGNS-1	K151AGNS-2	K151AGNS-4	K151AGNS-21	K151AGNS-22	K151AGNS-24
S-PLEX NHP Tau (pT231)	K156AGNS-1	K156AGNS-2	K156AGNS-4	K156AGNS-21	K156AGNS-22	K156AGNS-24

Plate Diagram

Figure 5 and Figure 6 are provided for illustration.

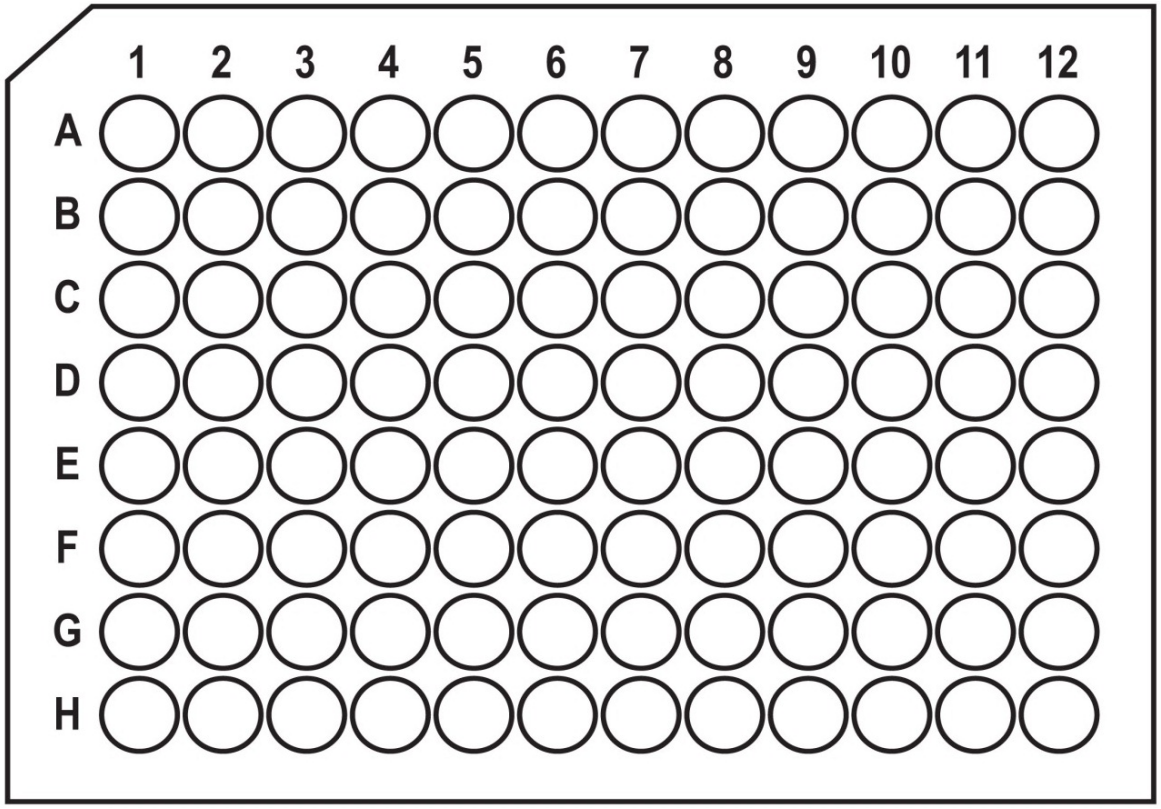

Figure 5. Plate diagram.

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