SPLASH™ Kit: NfL Singleplex

P/N: TSK-00018

Product Description

Taudia's SPLASH Kit: NfL Singleplex is designed to quantify neurofilament light chain (NfL) protein in biofluids using a qPCR instrument. The following protocol is designed to run 1 × 96-well plate; the kit includes sufficient materials to run a total of 96 wells across two runs.

Contents and storage

Store the contents according to the table below at 4 °C or -20 °C. Never freeze Magnetic Beads.

Items	Quantity	Storage
Standard A	2 vials	-20 °C
CheckPoint A	2 vials	
20x AmPLAmix Additive	300 µL	
AmPLAmix	5.7 mL	
Sample Buffer	8 mL	4 °C
Probe Buffer	6.5 mL	
Wash Buffer	50 mL	
Magnetic Beads	5 mL	
NfL Probe A	60 µL	
NfL Probe B	60 µL	

Required materials

Taudia's SPLASH Kits are designed to work on 96-well qPCR instruments in the FAM channel.

Equipment and Materials Required (not provided)

- 96-well qPCR Instrument (FAM)
- 96-well plate magnetic bead separation rack
- 96-well qPCR plate with at least 0.2 mL well volume
- Microtiter plate adhesive seal or aluminum foil seal
- Microtiter plate adhesive seal, optical grade
- Calibrated pipettes and low retention filter tips
- Tubes or similar plastics for dilutions; 15 mL tube
- Reagent reservoirs for multichannel pipettes (optional)
- Ice and ice bucket (optional)
- Vortex (optional)

Protocol

Step 1: Binding (15 mins + 2 hr incubation)

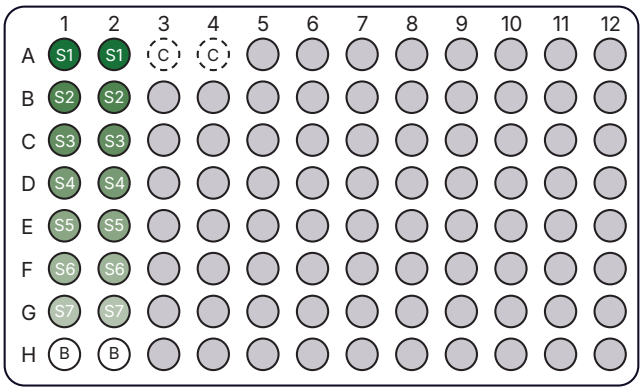
☐ ***Prepare and Plate Standard Curve*** ●●

For each run, reconstitute 1 vial of protein standard by adding 500 µL of Sample Buffer. Cap and invert the tube 5 times; do not vortex protein standards. Allow the vial to reconstitute at room temperature for 10 mins. Keep standards on ice or at 4 °C. To prepare the standard curve:

1. Label a tube "Blank" and add 120 µL of Sample Buffer.
2. Label tubes for 7 dilution points (i.e., S1, S2, ..., S7).
3. Add 120 µL of Sample Buffer to tubes S1 to S7.
4. Prepare S1 by adding 40 µL of Standard to the S1 tube. Pipette up and down 15-20 times to mix.
5. Prepare a 4X dilution of S1 by adding 40 µL of S1 to the S2 tube. Pipette up and down 15-20 times to mix.
6. Continue serial dilution to S7.
7. Plate 50 µL of each standard and blank into the 96-well plate as duplicates in Columns 1 and 2.
8. Keep plate on ice or at 4 °C after plating.

☐ ***Prepare and Plate CheckPoint (optional)*** ○

CheckPoint is a calibrator between Standards 4 and 5; it is an optional QC check. Reconstitute 1 vial of CheckPoint by adding 500 µL of Sample Buffer. Cap and invert the tube 5 times; do not vortex CheckPoint. Allow the vial to reconstitute at room temperature for 10 mins. Plate 50 µL into A3 and A4.



☐ *Prepare and Plate Samples*

Dilute each plasma or serum sample by 4X in Sample Buffer to a total volume of 50 µL (25-50X for CSF). Diluting directly in the 96-well plate, add 37.5 µL of Sample Buffer followed by 12.5 µL of sample. Pipette up and down 5 times to mix.

☐ *Prepare Probe Mix*

To make the Probe Mix for 96 samples, add the following components in the order listed to a 15 mL tube, using a new pipette tip for each probe. Mix by gently inverting the tube 5-10 times. Keep the Probe Mix on ice or at 4 °C.

Component	Volume
Probe Buffer	6000 µL
NfL Probe A	60 µL
NfL Probe B	60 µL

☐ *Prepare Binding Reactions*

Add 50 µL of Probe Mix directly to all plated wells.

☐ *Immunocomplex Formation*

Seal the 96-well plate with an adhesive seal. Seal will be removed after incubation. Incubate for 2 hours at room temperature (alternatively overnight at 4 °C).

☐ *Completely Thaw AmPLAmix and 20x AmPLAmix Additive During 2 hr Incubation*

Step 2: Washing (15 mins)

☐ *Add Magnetic Beads*

Thoroughly mix the Magnetic Beads until homogeneous by vigorously shaking for 30 seconds or vortex. Remove the seal from the 96-well plate and add 50 µL of Magnetic Beads into each reaction well. Pipette up and down 2 times to mix.

☐ *Pull Down Immunocomplexes*

Incubate for 5-10 minutes at room temperature from the last bead addition. During the bead incubation, proceed to the next step.

☐ *Add 20x AmPLAmix Additive to AmPLAmix*

Thoroughly mix the 20x AmPLAmix Additive by vigorously shaking for 30 seconds or vortex. For 96 samples, add 300 µL of the 20x AmPLAmix Additive to 5700 µL of AmPLAmix. Cap and invert the tube 5-10 times. Keep on ice or at 4 °C.

☐ *Wash Step*

Wash 1:

1. Place the 96-well plate onto a magnetic rack. Wait at least 1 minute to pull down the beads.
2. Decant the liquid (i.e., flip the plate while on the magnetic rack 3 times into a sink or biohazard receptacle; optionally use a pipette to decant).
3. Keep the plate on the magnetic rack and add 200 µL of Wash Buffer to each well.

Wash 2: Repeat steps 2-3 from Wash 1.

Post-wash: Decant the liquid. While on the magnetic rack, invert and tap the plate onto a paper towel to remove residual liquid.

Immediately proceed to the next step.

Step 3: qPCR (1 hr protocol)

☐ *qPCR Step**

1. **While still on the magnetic rack**, add 50 µL of AmPLAmix with Additive to each well.
2. Seal the 96-well plate with an optical adhesive seal.
3. Remove the 96-well plate from the magnetic rack.
4. **Within 15 mins of adding the AmPLAmix with Additive,** run the following qPCR protocol:

Step	Temp	Time	Stage
Ligation	30 °C	15 min	Hold
Ligase Inactivation	95 °C	3 min	Hold
Pre-Amplification	95 °C	30 s	3 Cycles
	60 °C	1 min	
PCR[1]	95 °C	15 s	37 Cycles
	60 °C	30 s	

[1] Turn on data collection in FAM.

Step 4: Analysis

☐ *Analysis**

Retrieve the data from the qPCR instrument and use its companion software to set thresholds and export the Cq values as a .CSV or .XLSX. Import the .CSV or .XLSX file into Taudia's TallyPro™ Software to return pg/mL values.

**qPCR and Analysis Templates can be found at https://taudia.com/splash-ultrasensitive-biomarker-kits/ in the Documentation section under each kit. For help, please reach out to hello@taudia.com*

Lot Information

Lot	Standard	NfL Conc. (pg/mL)
A889588	Standard 1 (S1)	200
	Standard 2 (S2)	50.0
	Standard 3 (S3)	12.5
	Standard 4 (S4)	3.13
	Standard 5 (S5)	0.781
	Standard 6 (S6)	0.195
	Standard 7 (S7)	0.049
	Blank (B)	0
	Optional - CheckPoint (C)	*0.781-3.13*