SPLASH™ Kit: NfL & GFAP Duplex

P/N: TSK-00008

Product Description

Taudia's SPLASH Kit: NfL and GFAP Duplex is designed to quantify neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) proteins 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 materials at 4 °C or -20 °C. Never freeze Magnetic Beads.

Items	Quantity	Storage
NfL & GFAP Standard	2 vials	-20 °C
CheckPoint	2 vials	
NfL & GFAP AmPLAmix	5 mL	
Sample Buffer	6.5 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	
GFAP Probe A	60 µL	
GFAP Probe B	60 µL	

Required materials

Taudia's SPLASH Kits (Duplex) are designed to work on 96-well qPCR instruments in the FAM and VIC/HEX channels.

Equipment and Materials Required (not provided)

- 96-well qPCR Instrument (FAM and VIC/HEX Channels)
- 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)

Protocol

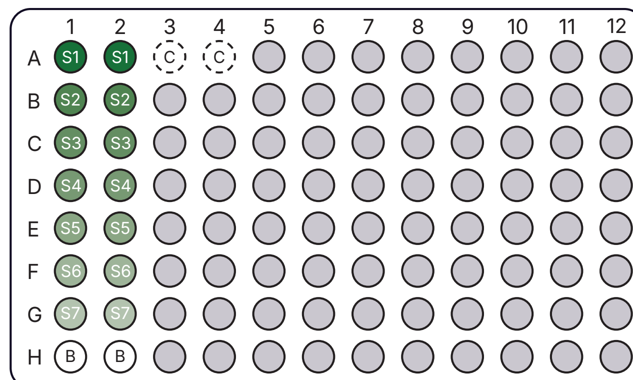
Step 1: Binding (15 mins + 2 hr incubation)

☐ ***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 10 times to mix.
5. Prepare a 4X dilution of S1 by adding 40 µL of S1 to the S2 tube. Pipette up and down 10 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.

Standard	NfL Conc. (pg/mL)	GFAP Conc. (pg/mL)
Standard 1 (S1)	400	10000
Standard 2 (S2)	100	2500
Standard 3 (S3)	25	625
Standard 4 (S4)	6.25	156.3
Standard 5 (S5)	1.56	39.06
Standard 6 (S6)	0.391	9.77
Standard 7 (S7)	0.098	2.44
Blank (B)	0	0
Optional - CheckPoint (C)	1.56-6.25	39.06-156.3



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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. 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 12.5 µL of sample and 37.5 µL of Sample Buffer.

Prepare Probe Mix

For 96 samples, add the following components in the order listed to a 15 mL tube. 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
GFAP Probe A	60 µL
GFAP 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).

Thaw AmPLAmix on Ice During 2 hr Incubation

Step 2: Washing (15 mins)

Add Magnetic Beads

Thoroughly mix the Magnetic Beads until homogenous by shaking for 10-15 seconds or vortex. Remove the seal from the 96-well plate and add 50 µL of Magnetic Beads into each reaction well.

Pull Down Immunocomplexes

Incubate for 5 minutes at room temperature from the last bead addition.

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 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 adding the AmPLAmix**, run the following qPCR protocol:

Step	Temp	Time	Stage
Ligation	30 °C	15 min	Hold
Ligase Inactivation	95 °C	3 min	Hold
PCR[1]	95 °C	10 s	40 Cycles
	60 °C	30 s	

[1] Turn on data collection in FAM for NfL and VIC/HEX for GFAP.

Step 4: Analysis

Analysis

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