SPLASH™ Kit: Aβ40 & Aβ42 Duplex

P/N: TSK-00007

Product Description

Taudia's SPLASH Kit: Aβ40 & Aβ42 Duplex is designed to quantify amyloid-β 1-40 (Aβ40) and amyloid-β 1-42 (Aβ42) 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
Aβ40 & Aβ42 Standard	2 vials	-20 °C
CheckPoint	2 vials	
Aβ40 & Aβ42 AmPLAmix	5 mL	
Sample Buffer	6.5 mL	4 °C
Probe Buffer	6.5 mL	
Wash Buffer	50 mL	
Magnetic Beads	5 mL	
Aβ40 Probe A	60 µL	
Aβ Probe B	60 µL	
Aβ42 Probe A	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

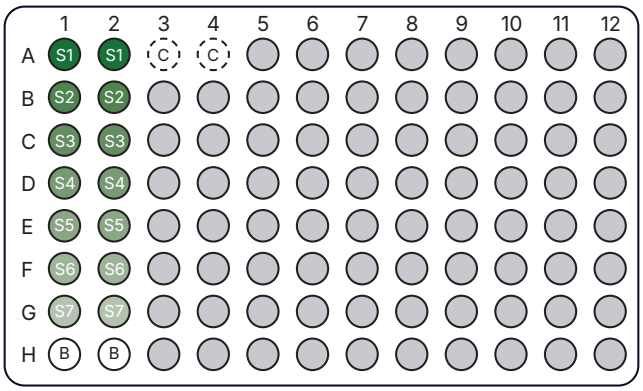
Step 1: Binding (15 mins + 2 hr incubation)

☐ ***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 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.

Standard	Aβ40 Conc. (pg/mL)	Aβ42 Conc. (pg/mL)
Standard 1 (S1)	2000	400
Standard 2 (S2)	500	100
Standard 3 (S3)	125	25.0
Standard 4 (S4)	31.3	6.25
Standard 5 (S5)	7.81	1.56
Standard 6 (S6)	1.95	0.391
Standard 7 (S7)	0.488	0.098
Blank (B)	0	0
Optional - CheckPoint (C)	7.81-31.3	1.56-6.25



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 37.5 µL of Sample Buffer and 12.5 µL of sample. Pipette up and down 5 times to mix.

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
Aβ40 Probe A	60 µL
Aβ Probe B	60 µL
Aβ42 Probe A	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 at 4 °C During 2 hr Incubation

Step 2: Washing (15 mins)

Add Magnetic Beads

Thoroughly mix the Magnetic Beads until homogenous 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.

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
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 for Aβ40 and VIC/HEX for Aβ42.

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 TallyPro™ Software to return pg/mL values.

**qPCR and Analysis Templates can be found at www.taudia.com/shop under each kit. For more information, please reach out to hello@taudia.com*