Differential Scanning Fluorimetry

Thermal shift assay of a protein with TFA and PFOA in a 384-well plate, 10 µL per well, in quintuplicate. Adapted from Bai et al., STAR Protocols.

Materials

- Purified protein (20 µM working stock)
- HBS (140 mM NaCl, 25 mM HEPES, 0.38 mM K_2HPO_4, pH 7.4)
- DMSO and glycerol
- 99% TFA
- PFOA, 95% (Sigma 171468)
- SYPRO Orange, 5000X

Protect all SYPRO Orange solutions from light.

Equipment

- qPCR instrument with FRET channel
- 384-well qPCR plate and optical seal
- PCR strips (8 tubes) and strip caps
- Multichannel pipette and tips
- pH meter
- Analytical balance
- Plate centrifuge

Buffers and Ligand Stocks

Solution	Preparation	Storage
HBS, 500 mL	140 mM NaCl, 25 mM HEPES, 0.38 mM K_2HPO_4, pH 7.4	4 °C
Buffer A, 100 mL	99.0 mL HBS + 1.0 mL DMSO (HBS + 1% DMSO)	4 °C
Buffer B, 100 mL	94.0 mL HBS + 1.0 mL DMSO + 5.0 mL glycerol (HBS + 1% DMSO + 5% glycerol)	4 °C
TFA, 50 mM, 10 mL	38.69 µL 99% TFA + 100 µL DMSO; adjust to pH 7.40 and bring to 10.00 mL with HBS	−20 °C, 1 mL aliquots
TFA, 5 mM, 10 mL	1.00 mL 50 mM TFA + 9.00 mL Buffer A	−20 °C, 1 mL aliquots
PFOA, 50 mM, 10 mL	217.93 mg Sigma 171468 (95%) + 100 µL DMSO; adjust to pH 7.40 and bring to 10.00 mL with HBS	−20 °C, 1 mL PP aliquots
PFOA, 5 mM, 10 mL	1.00 mL 50 mM PFOA + 9.00 mL Buffer A	−20 °C, 1 mL PP aliquots
PFOA, 1 mM, 10 mL	2.00 mL 5 mM PFOA + 8.00 mL Buffer A	−20 °C, 1 mL PP aliquots

Protein and Dye Working Solutions

Working solution	Preparation	Final in well
Protein, 4X	Dilute the purified protein to 20 µM in Buffer B	5 µM
50X SYPRO, 250 µL	2.5 µL 5000X SYPRO Orange + 247.5 µL Buffer A	–
10X SYPRO, 1.10 mL	220 µL 50X SYPRO + 880 µL Buffer A	5X

Store the 5000X SYPRO Orange stock at −25 to −30 °C, protected from light

4X Ligand Strips

Strip 1 – TFA

Final (mM)	4X (mM)	Source	Src (µL)	Buf A (µL)
0.10	0.40	5 mM	4.8	55.2
0.30	1.20	5 mM	14.4	45.6
1.0	4.0	50 mM	4.8	55.2
2.0	8.0	50 mM	9.6	50.4
3.0	12.0	50 mM	14.4	45.6
5.0	20.0	50 mM	24	36
7.5	30.0	50 mM	36	24
10.0	40.0	50 mM	72	18*

Strip 2 – PFOA

Final (mM)	4X (mM)	Source	Src (µL)	Buf A (µL)
0.03	0.12	1 mM	7.2	52.8
0.06	0.24	1 mM	14.4	45.6
0.10	0.40	5 mM	4.8	55.2
0.20	0.80	5 mM	9.6	50.4
0.30	1.20	5 mM	14.4	45.6
0.50	2.00	5 mM	24	36
1.0	4.00	5 mM	48	12
3.0	12.00	50 mM	21.6	68.4*

* Every tube is 60 µL total, except the last of each strip, which is 90 µL

Reaction Strips

Strips 3 and 4 follow the order of strip 1 (TFA); strips 5 and 6 follow the order of strip 2 (PFOA). Mix gently before and after the incubation, and avoid bubbles.

Strip	Per reaction tube	After 20 min at RT	Dispense
3, 5 (protein)	24 µL 20 µM protein + 24 µL matching 4X ligand	add 48 µL 10X SYPRO	10 µL × 4 wells
4, 6 (no protein)	24 µL Buffer B + 24 µL matching 4X ligand	add 48 µL 10X SYPRO	10 µL × 4 wells

Control Strip 7

Tube	Buf A (µL)	Buf B (µL)	10X SYPRO (µL)	40 mM TFA (µL)	12 mM PFOA (µL)	4X protein (µL)
C1 Medium only	72	24	–	–	–	–
C2 Medium + dye	24	24	48	–	–	–
C3 Protein only	72	–	–	–	–	24
C4 Protein + dye	24	–	48	–	–	24
C5 TFA, no dye	48	24	–	24	–	–
C6 PFOA, no dye	48	24	–	–	24	–

Plate Reader Settings

Read SYPRO Orange on the FRET channel, excitation 490 nm, emission 600 nm.

Step	RFU read	Temp	Time	ΔTemp	Cycles
1	yes	25 °C	10 s	+0.5 °C per cycle	70
2	no	25 °C	hold	–	–

Final concentrations in matched wells: 5 µM protein, 5X SYPRO Orange, 1% DMSO and 1.25% glycerol. Dispense 10–12 µL per well in the desired plate layout, seal, and spin the plate briefly before the run