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αS PFF Assembly Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details the α S PFF assembly.

Attachments



Generation of recomb...

22KB

Troubleshooting



Aggregation

- 1 Remove monomer stock from -80 °C and thaw On ice .
- 2 Dilute stock to desired concentration (typically 5 µL) to a final volume of 300 µL in autoclaved 2 mL centrifuge tubes.
- 3 Set thermocycler for continuous shaking (1000 rpm, 37°C).
- 4 Install 2 mL plate.
- 5 Run PBS and Protein samples. Take 2 µL aliquot at 0 hr time point. Run ThioT Assay.
- 6 Take 2 µL aliquots twice per day for up to 120:00:00 (72 hours - 96 hours may be sufficient).
- 7 Check ThioT Fluorescence.
- 8 Aliquot and store at -80 °C .

ThioT Assay

- 9 Prepare ThioT stock mixture (8 µg ThioT in 10 µL milliQ H₂O).
- 10 Prepare working ThioT mixture, 20 µL stock into 1 mL PBS.
- 11 Dispense 200 µL working solution into each 2 µL aliquot.
- 12 Dispense 200 µL per sample into 96-well, black, corning glass-bottomed plate.



13 Read Fluorescence Intensity (Ex 450 +/-10; Em 490 +/- 10).