CLAIMS

1. An in vitro method for detecting the presence of soluble, misfolded α-synuclein (α-syn) protein in a biological sample, the method comprising:

(A) providing a biological sample;

(B) providing a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α-syn protein;

(2) a buffer composition;

(3) a salt;

(4) a fluorescent protein aggregation indicator; and

(5) one or more Si_3N_4 beads having a diameter of 1 mm to 5 mm;

(C) combining the biological sample and the pre-incubation mixture to form an incubation mixture;

(D) incubating and shaking the incubation mixture a number of times to form an incubated mixture;

(E) illuminating the incubated mixture with a wavelength of light that excites the fluorescent protein aggregation indicator; and

(F) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence during incubation indicates the presence of soluble, misfolded α-syn protein in the biological sample.

2. The method of claim 1, wherein the Si3N4 bead has a diameter of 2.3 mm to 5 mm.

3. The method of claim 1, wherein the monomeric α-syn protein is present in a concentration of from 10 μM±10% to 30 μM±10%.

4. The method of claim 1, wherein the monomeric α-syn protein comprises SEQ ID NO. 2.

5. The method of claim 1, wherein the buffer composition has a pH of between 6.2±10% to 6.5±10%.

6. The method of claim 1, wherein the buffer composition comprises PIPES.

7. The method of claim 1, wherein the salt comprises NaCl.

8. The method of claim 1, wherein the salt comprises NaCl in a concentration between 500±10% mM to 700±10% mM.

9. The method of claim 1, wherein the fluorescent protein aggregation indicator comprises thioflavin T (ThT).

10. An in vitro method for detecting the presence of soluble, misfolded α-synuclein (α-syn) protein in a biological sample, the method comprising:

(A) providing a biological sample;

(B) providing a pre-incubation mixture, the pre-incubation mixture comprising:

(1) a monomeric α-syn protein;

(2) a buffer composition;

(3) a salt;

(4) a fluorescent protein aggregation indicator; and

(5) a borosilicate glass bead having a diameter greater than 2.3 mm;

(C) combining the biological sample and the pre-incubation mixture to form an incubation mixture;

(D) incubating and shaking the incubation mixture a number of times to form an incubated mixture;

(E) illuminating the incubated mixture with a wavelength of light that excites the protein aggregation indicator; and

(F) determining a level of fluorescence during incubation, wherein an increase in the level of fluorescence during incubation indicates the presence of soluble, misfolded α-syn protein in the biological sample.

11. The method of claim 10, wherein:

(i) the monomeric α-syn protein is present in a concentration of from 10 μM±10% to 30 μM±10%;

(ii) the buffer composition comprises PIPES and has a pH of from 6.2±10% to 6.5±10%;

(iii) the salt comprises NaCl in a concentration of from 500±10% mM to 700 mM±10%;

(iv) the borosilicate glass bead has a diameter of 2.45 mm±10%;

(v) the fluorescent protein aggregation indicator comprises thioflavin T (ThT) in a concentration of from 5 μM to 10 μM; and

(vi) the detection comprises measuring ThT fluorescence.

12. The method of claim 1, wherein the pre-incubation mixture further comprises sarkosyl.

13. The method of claim 10, wherein the pre-incubation mixture further comprises sarkosyl.

14. The method of claim 1, wherein the pre-incubation mixture further comprises sarkosyl in a concentration of 0.1% w/v in IX PBS.

15. The method of claim 10, wherein the monomeric α-syn protein comprises SEQ ID NO: 2.