

protein concentration in the test sample is from 2 µg/mL to 100 µg/mL. In some examples, the total protein concentration in the test sample is from 2 µg/mL to 60 µg/mL. In some such examples, the total protein concentration of the test sample or of the biological sample is adjusted to the range of from about 2 µg/mL to about 60 µg/mL following determination that the total protein concentration is not in the range. In some examples, the total protein concentration of the test sample is in the range of from about 2 µg/mL to about 60 µg/mL without an adjustment of total protein concentration. In some such examples, the total protein concentration is measured using a fluorescence readout assay. In some examples, the total protein concentration in the test sample is from 70 µg/mL to 100 µg/mL. In some such examples, the total protein concentration of the test sample is adjusted to the range of from about 70 µg/mL to about 100 µg/mL following determination that the total protein concentration is not in the range. In some examples, the total protein concentration of the test sample is in the range of from about 70 µg/mL to about 100 µg/mL without an adjustment of total protein concentration. In some such examples, the total protein concentration is measured using a BCA assay. In some examples, the total protein concentration of each of a plurality of test samples or of a plurality of biological samples is adjusted to be the same.

In one aspect, the present disclosure refers to a method for preparing a plurality of biological samples for detecting a protein comprising:

generating a plurality of test samples by performing a buffer exchange on the plurality of biological samples by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant, and generating a plurality of adjusted test samples by adjusting the total protein concentrations of a plurality of test samples, wherein the total protein concentration of each adjusted test sample is from 70 µg/mL to 100 µg/mL; wherein the method does not comprise concentrating the total protein of the plurality of biological samples prior to performing the buffer exchange; and wherein the plurality of biological samples are urine samples.

In one aspect, the present disclosure refers to a method for preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70 µg/mL to 100 µg/mL; and wherein the biological sample is a urine sample.

In one aspect, the present disclosure refers to a method for preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts,
5 a chelating agent and a nonionic surfactant;

determining the total protein concentration of the test sample; and

generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and

10 wherein the biological sample is a urine sample.

In one aspect, the present disclosure refers to a method of preparing a biological sample for detecting a protein comprising:

generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts,
15 a chelating agent and a nonionic surfactant;

determining the protein concentration of the test sample; and

if the total protein concentration of the test sample is not in the range of from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$, generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70
20 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and

wherein the biological sample is a urine sample.

In one aspect, the present disclosure refers to a method for detecting a target protein in a test sample comprising:

a) contacting the test sample with at least one capture reagent, wherein the at least one

25 capture reagent is capable of binding to the target protein to form a complex;

b) incubating the test sample with the at least one capture reagent under conditions that allow for the complex to form; and

c) determining the level of the target protein in the test sample by measuring the level of the at least one capture reagent, the complex, or the protein; wherein the level of the at least one

30 capture reagent or the complex is a surrogate for the level of the target protein;

wherein, the test sample is generated by performing a buffer exchange of a biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, a salt, a chelating agent and a nonionic surfactant; and

wherein, the total protein concentration of the test sample is 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and wherein the test sample is a urine sample.

Certain examples of embodiments provided herein are listed below.

Embodiment 1. A method for preparing a biological sample for detecting a protein comprising:

a) generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and

b) generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from about 2 $\mu\text{g/mL}$ to about 60 $\mu\text{g/mL}$.

Embodiment 2. The method of embodiment 1 comprising determining the protein concentration of the test sample.

Embodiment 3. A method for preparing a biological sample for detecting a protein comprising:

a) generating a test sample by performing a buffer exchange on the biological sample using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant;

b) determining the total protein concentration of the test sample; and

LISTING OF CLAIMS

1. A method for preparing a plurality of biological samples for detecting a protein comprising:
 - generating a plurality of test samples by performing a buffer exchange on the plurality of biological samples by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant, and
 - generating a plurality of adjusted test samples by adjusting the total protein concentrations of a plurality of test samples, wherein the total protein concentration of each adjusted test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$;
 - wherein the method does not comprise concentrating the total protein of the plurality of biological samples prior to performing the buffer exchange; and
 - wherein the plurality of biological samples are urine samples.
2. A method for preparing a biological sample for detecting a protein comprising:
 - generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and
 - generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and
 - wherein the biological sample is a urine sample.
3. The method of claim 1 or 2 comprising determining the protein concentration of the test sample or plurality of test samples and/or the biological sample or plurality of biological samples.
4. A method for preparing a biological sample for detecting a protein comprising:
 - generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant;
 - determining the total protein concentration of the test sample; and
 - generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and

wherein the biological sample is a urine sample.

5. The method of any one of claims 2-4, comprising generating a plurality of test samples by performing a buffer exchange on a plurality of biological samples, wherein the total protein concentration of each adjusted test sample is about the same.

6. The method of any one of claims 1-5, comprising generating a plurality of test samples by performing a buffer exchange on a plurality of biological samples, wherein the total protein concentration of each adjusted test sample is the same.

7. A method of preparing a biological sample for detecting a protein comprising:
generating a test sample by performing a buffer exchange on the biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant;

determining the protein concentration of the test sample; and

if the total protein concentration of the test sample is not in the range of from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$, generating an adjusted test sample by adjusting the total protein concentration of the test sample, wherein the total protein concentration in the adjusted test sample is from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and

wherein the biological sample is a urine sample.

8. The method of claim 7, wherein if the total protein concentration of the test sample is not in the range of from 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; or from 70 $\mu\text{g/mL}$ to 95 $\mu\text{g/mL}$; or from 70 $\mu\text{g/mL}$ to 90 $\mu\text{g/mL}$; or from 70 $\mu\text{g/mL}$ to 85 $\mu\text{g/mL}$; or from 70 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$; or from 70 $\mu\text{g/mL}$ to 75 $\mu\text{g/mL}$, generating an adjusted test sample by adjusting the total protein concentration of the test sample to the range.

9. The method of any one of claims 2-8, wherein the method does not comprise concentrating the biological sample.

10. A method for detecting a target protein in a test sample comprising:

a) contacting the test sample with at least one capture reagent, wherein the at least one capture reagent is capable of binding to the target protein to form a complex;

b) incubating the test sample with the at least one capture reagent under conditions that allow for the complex to form; and

c) determining the level of the target protein in the test sample by measuring the level of the at least one capture reagent, the complex, or the protein; wherein the level of the at least one capture reagent or the complex is a surrogate for the level of the target protein; wherein, the test sample is generated by performing a buffer exchange of a biological sample by exchanging a starting buffer with a formulation comprising a buffering agent, a salt, a chelating agent and a nonionic surfactant; and wherein, the total protein concentration of the test sample is 70 $\mu\text{g/mL}$ to 100 $\mu\text{g/mL}$; and wherein the test sample is a urine sample.

11. The method of claim 10, wherein the at least one protein capture reagent is an aptamer or an antibody.

12. The method of claim 10 or 11 comprising a plurality of capture reagents, where each capture reagent is an aptamer.

13. The method of any one of claims 1-12, wherein the total protein concentration of the test sample is 70 $\mu\text{g/mL}$ to 95 $\mu\text{g/mL}$; or 70 $\mu\text{g/mL}$ to 90 $\mu\text{g/mL}$; or 70 $\mu\text{g/mL}$ to 85 $\mu\text{g/mL}$; or 70 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$; or 70 $\mu\text{g/mL}$ to 75 $\mu\text{g/mL}$ or 70 $\mu\text{g/mL}$.

14. The method of any one of claims 1-13, wherein the one or more salts are selected from a sodium salt, a potassium salt and a magnesium salt.

15. The method of any one of claims 1-13, wherein the one or more salts comprise a sodium salt, a potassium salt and a magnesium salt.

16. The method of claim 14 or 15, wherein the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl_2 .

17. The method of claim 16, wherein the NaCl in the formulation is at a concentration of from 10 mM to 500 mM, or from 50 mM to 250 mM, or from 100 mM to 200 mM or 102 mM.

18. The method of claim 16 or 17, wherein the KCl in the formulation is at a concentration of from 0.5 mM to 30 mM, or from 1 mM to 20 mM, or from 2 mM to 15 mM, or from 4 mM to 10 mM, or 5 mM.

19. The method of any one of claims 16-18, wherein the MgCl_2 in the formulation is at a concentration of from 0.5 mM to 30 mM, or from 1 mM to 20 mM, or from 2 mM to 15 mM, or from 4 mM to 10 mM, or 5 mM.

20. The method of any one of claims 1-19, wherein the buffering agent is selected from HEPES, MES, Bis-tris methane, ADA, ACES, Bis-tris propane, PIPES, MOPSO, Choline

chloride, MOPS, BES, TES, DIPSO, MOB, Acetamidoglycine, TAPSO, TEA, POPSO, HEPPSO, EPS, HEPPS, Tricine, Tris, Glycinamide, Glycylglycine, HEPBS, Bicine, TAPS, AMPB, CHES, AMP, AMPSO, CAPSO, CAPS and CABS.

21. The method of any one of claims 1-20, wherein the buffering agent in the formulation is at a concentration of from 4 mM to 400 mM, or from 10 mM to 300 mM, or from 20 mM to 200 mM, or from 30 mM to 100 mM, or from 35 mM to 50 mM or 40 mM.

22. The method of any one of claims 1-21, wherein the chelating agent is selected from EDTA, EGTA, DTPA, BAPTA, DMPS and ALA.

23. The method of any one of claims 1-22, wherein the chelating agent in the formulation is at a concentration of 0.1 mM to 10 mM, or from 0.5 mM to 5 mM, or 1 mM.

24. The method of any one of claims 1-23, wherein the nonionic surfactant is selected from Polyoxyethylene (20) sorbitan monolaurate (Tween-20), Polyoxyethylene (40) sorbitan monolaurate (Tween-40) and Polyoxyethylene (80) sorbitan monolaurate (Tween-80).

25. The method of any one of claims 1-24, wherein the nonionic surfactant is from 0.01% to 1% of the formulation, or 0.02% to 0.5% of the formulation, or from 0.03% to 0.1% of the formulation, or from 0.04% to 0.08% of the formulation, or 0.05% of the formulation.

26. The method of any one of claims 1-25, wherein the formulation comprises 40 mM HEPES, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 0.05% Tween 20.

27. The method of any one of claims 1-25, wherein the formulation consists of 40 mM HEPES, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 0.05% Tween 20 at pH 7.5.

28. The method of any one of claims 1-26, wherein the pH of the formulation is from pH 5 to pH 9, or from pH 6 to pH 8, or from pH 7 to pH 7.9, or pH 7.5.

29. The method of any one of claims 11-28, wherein the aptamer comprises a 5-position modified pyrimidine.

30. The method of claim 29, wherein the 5-position modified pyrimidine comprises a hydrophobic moiety at the 5-position of the pyrimidine.

31. The method of claim 30, wherein the hydrophobic moiety is selected from a naphthyl moiety, a phenyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety.

32. The method of claim 30 or 31, wherein the hydrophobic moiety is covalently linked to the 5-position of the based via a linker comprising a group selected from an amide linker, a

carbonyl linker, a propynyl linker, an alkyne linker, an ester linker, a urea linker, a carbamate linker, a guanidine linker, an amidine linker, a sulfoxide linker, and a sulfone linker.

33. The method of any one of claims 10-32, wherein the complex is a non-covalent complex.

34. The method of any one of claims 10-33, wherein the at least one capture reagent is attached to a first solid support before contacting the test sample or the capture reagent is attached to a first solid support after contacting the test sample.

35. The method of any one of claims 10-34, further comprising attaching the complex to a first solid support via the capture reagent and releasing complex from the first solid support and attaching the complex to a second solid support, wherein the complex is attached to the second solid support via the protein.

36. The method of any one of claims 10-35, further comprising adding a competitor molecule to the test sample and/or adding a competitor molecule and diluting the test sample.

37. The method of claim 36, wherein the competitor molecule is a polyanionic competitor.

38. The method of claim 37, wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin and dNTPs.

39. The method of claim 38, wherein the polyanionic competitor is a polydextran, and wherein the polydextran is dextran sulfate or wherein the polyanionic competitor is an oligonucleotide, and wherein the oligonucleotide comprises one or more 5-position modified pyrimidines.

40. The method of any one of claims 1-39, wherein the buffer exchange is not performed with ultrafiltration.

41. The method of any one of claims 1-40, wherein the buffer exchange is performed using gel filtration chromatography.

42. The method of any one of claims 1-41, comprising measuring the protein concentration of the biological sample prior to the performing of the buffer exchange.

43. The method of claim 42, wherein the protein concentration of the biological sample is measured with an assay selected from the group consisting of a fluorescence readout assay, a bicinchoninic acid (BCA) assay, a micro BCA assay, a Lowry assay, and an ELISA assay.

44. The method of claim 43, wherein the assay is a BCA assay or a micro BCA assay.

45. The method of claim 44, wherein the assay comprises a bicinchoninic acid reagent or a bicinchonic acid reagent and, optionally, an alkaline tartrate-carbonate buffer and/or a copper sulfate solution.
46. The method of claim 43, wherein the assay is a fluorescence readout assay, optionally wherein the fluorescence readout assay comprises a reagent selected from a merocyanine dye and epicocconone.