

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 ~~using~~ 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 ~~about the same~~;

wherein the method does not comprise concentrating the total protein of the plurality of biological samples prior to performing the buffer exchange.

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 ~~using~~ 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 ~~about~~ 70 µg/mL to ~~about~~ 100 µg/mL.

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 ~~using~~ 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 ~~about~~ 70 $\mu\text{g/mL}$ to ~~about~~ 100 $\mu\text{g/mL}$.

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 ~~using~~ 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 ~~about~~ 70 $\mu\text{g/mL}$ to ~~about~~ 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 ~~about~~ 70 $\mu\text{g/mL}$ to ~~about~~ 100 $\mu\text{g/mL}$.

~~In one aspect, the present disclosure refers to a test sample comprising a buffer-exchanged biological sample, wherein the test sample has a total protein concentration from about 70 $\mu\text{g/mL}$ to about 100 $\mu\text{g/mL}$, and wherein the buffer-exchanged biological sample comprises a buffering agent, one or more salts, a chelating agent and a nonionic surfactant.~~

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 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 ~~about 70 µg/mL~~ to ~~about less than~~ 100 µg/mL

~~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 using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and~~

~~—determining the total protein concentration of the biological sample or of the test sample; and~~

~~—adjusting the total protein concentration of the biological sample or of the test sample, wherein the method does not comprise concentrating the total protein of the biological sample prior to performing the buffer exchange;~~

~~—optionally wherein (i) the total protein concentration is adjusted before performing the buffer exchange or (ii) the total protein concentration is adjusted after performing the buffer exchange.~~

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 µg/mL to about 60 µg/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 ~~using~~ 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 ~~about the same~~;

wherein the method does not comprise concentrating the total protein of the plurality of biological samples prior to performing the buffer exchange.

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 ~~using~~ 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 ~~about~~ 70 µg/mL to ~~about~~ 100 µg/mL.

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 ~~using~~ 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 ~~about~~ 70 µg/mL to ~~about~~ 100 µg/mL.

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 ~~using~~
~~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 ~~about~~ 70 µg/mL to ~~about~~ 100 µg/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 ~~about~~ 70 µg/mL to ~~about~~ 100 µg/mL.

8. The method of claim 7, wherein if the total protein concentration of the test sample is not in the range of from ~~about~~ 70 µg/mL to ~~about~~ 100 µg/mL; or from ~~about~~ 70 µg/mL to ~~about~~ 95 µg/mL; or from ~~about~~ 70 µg/mL to ~~about~~ 90 µg/mL; or from ~~about~~ 70 µg/mL to ~~about~~ 85 µg/mL; or from ~~about~~ 70 µg/mL to ~~about~~ 80 µg/mL; or from ~~about~~ 70 µg/mL to ~~about~~ 75 µg/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 1-8, wherein the one or more salts are each independently selected from a sodium salt, a potassium salt and a magnesium salt.~~

~~10. — The method of any one of claims 1-8, wherein the one or more salts comprise a sodium salt, a potassium salt and a magnesium salt.~~

~~11. — The method of any one of claims 9 or 10, wherein the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl₂.~~

~~12. — The method of claim 11, wherein the NaCl in the formulation is at a concentration of from about 10 mM to about 500 mM, or from about 50 mM to about 250 mM, or from about 100 mM to about 200 mM, or from about 75 mM to about 125 mM, or about 102 mM.~~

13. —The method of claim 11 or 12, wherein the KCl in the formulation is at a concentration of from about 0.5 mM to about 30 mM, or from about 1 mM to about 20 mM, or from about 2 mM to about 15 mM, or from about 4 mM to about 10 mM, or about 5 mM.

14. —The method of any one of claims 11–13, wherein the MgCl₂ in the formulation is at a concentration of from about 0.5 mM to about 30 mM, or from about 1 mM to about 20 mM, or from about 2 mM to about 15 mM, or from about 4 mM to about 10 mM, or about 5 mM.

15. —The method of any one of claims 1–14, 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.

16. —The method of claim 15, wherein the buffering agent in the formulation is at a concentration of from about 4 mM to about 400 mM, or from about 10 mM to about 300 mM, or from about 20 mM to about 200 mM, or from about 30 mM to about 100 mM, or from 35 mM to about 50 mM, or about 40 mM.

17. —The method of any one of claims 1–16, wherein the chelating agent is selected from EDTA, EGTA, DTPA, BAPTA, DMPS and ALA.

18. —The method of claim 17, wherein the chelating agent in the formulation is at a concentration of about 0.1 mM to about 10 mM, or from about 0.5 mM to about 5 mM, or about 1 mM.

19. —The method of any one of claims 1–18, 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).

20. —The method of claim 19, wherein the nonionic surfactant is from about 0.01% to about 1% of the formulation, or about 0.02% to about 0.5% of the formulation, or from about 0.03% to about 0.1% of the formulation, or from about 0.04% to about 0.08% of the formulation, or about 0.05%, or about 0.1% of the formulation, volume for volume.

21. —The method of any one of claims 1–20, wherein the total protein concentration of the adjusted test sample is from about 2 µg/mL to about 70 µg/mL; or from about 2 µg/mL to about 75 µg/mL; or from about 4 µg/mL to about 70 µg/mL; or from about 8 µg/mL to about 70 µg/mL; or from about 10 µg/mL to about 70 µg/mL; or from about 10 µg/mL to about 70.

22. — ~~The method of any one of claims 3-21, wherein the total protein concentration of the test sample or the adjusted test sample is determined with a bicinchoninic acid (BCA) assay, wherein the BCA assay is optionally a micro-BCA assay.~~

23. — ~~The method of claim 22, 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.~~

24. — ~~The method of any one of claims 1-23, wherein the formulation comprises 40 mM HEPES, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 0.05% Tween-20.~~

25. — ~~The method of claim any one of claims 1-24, wherein the pH of the formulation is from about pH 5 to about pH 9, or from about pH 6 to about pH 8, or from about pH 7 to about pH 7.9, or about pH 7.5.~~

26. — ~~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 and has a pH of 7.5.~~

27. — ~~The method of any one of claims 1-26, wherein the buffer exchange is not performed with ultrafiltration.~~

28. — ~~The method of any one of claims 1-27, wherein the buffer exchange is performed with gel filtration chromatography.~~

29. — ~~The method of any one of claims 1-28, wherein the biological sample is a urine sample.~~

30. — ~~The method of any one of claims 1-28, wherein the biological sample is a serum sample.~~

~~31-9.~~ 31.9. ~~The method of any one of claims 2-308,~~ wherein the method does not comprise ~~a~~ concentrating the biological sample.

32. — ~~A test sample comprising a buffer-exchanged biological sample, wherein the test sample has a total protein concentration from about 70 µg/mL to about 100 µg/mL, and wherein the buffer-exchanged biological sample comprises a buffering agent, one or more salts, a chelating agent and a nonionic surfactant.~~

33. — ~~The test sample of claim 32, further comprising one or more protein capture reagents.~~

34. — ~~The test sample of claim 33, wherein each of the one or more protein capture reagents is an aptamer or an antibody.~~

35. — ~~The test sample of any one of claims 32-34, wherein the total protein concentration of the test sample is from about 70 µg/mL to 95 µg/mL; or from about 70 µg/mL to 90 µg/mL; or from~~

about 70 $\mu\text{g/mL}$ to 85 $\mu\text{g/mL}$; or from about 70 $\mu\text{g/mL}$ to 80 $\mu\text{g/mL}$; or from about 70 $\mu\text{g/mL}$ to 75 $\mu\text{g/mL}$; or is about 70 $\mu\text{g/mL}$.

36. — The test sample of any one of claims 32-35, wherein the one or more salts are each independently selected from a sodium salt, a potassium salt and a magnesium salt.

37. — The test sample of any one of claims 32-36, wherein the one or more salts comprise a sodium salt, a potassium salt and a magnesium salt.

38. — The test sample of claim 36 or 37, wherein the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl_2 .

39. — The test sample of claim 38, wherein the NaCl in the test sample is at a concentration of from about 10 mM to about 500 mM, or from about 50 mM to about 250 mM, or from about 100 mM to about 200 mM or about 102 mM.

40. — The test sample of claim 38 or 39, wherein the KCl in the test sample is at a concentration of from about 0.5 mM to about 30 mM, or from about 1 mM to about 20 mM, or from about 2 mM to about 15 mM, or from about 4 mM to about 10 mM, or about 5 mM.

41. — The test sample of any one of claims 38-40, wherein the MgCl_2 in the test sample is at a concentration of from about 0.5 mM to about 30 mM, or from about 1 mM to about 20 mM, or from about 2 mM to about 15 mM, or from about 4 mM to about 10 mM, or about 5 mM.

42. — The test sample of any one of claims 32-41, 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.

43. — The test sample of any one of claims 32-42, wherein the buffering agent in the test sample is at a concentration of from about 4 mM to about 400 mM, or from about 10 mM to about 300 mM, or from about 20 mM to about 200 mM, or from about 30 mM to about 100 mM, or from 35 mM to about 50 mM or about 40 mM.

44. — The test sample of any one of claims 32-43, wherein the chelating agent is selected from EDTA, EGTA, DTPA, BAPTA, DMPS and ALA.

45. — The test sample of any one of claims 32-44, wherein the chelating agent in the test sample is at a concentration of about 0.1 mM to about 10 mM, or from about 0.5 mM to about 5 mM, or about 1 mM.

46. —The test sample of any one of claims 32-45, 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).

47. —The test sample of any one of claims 32-46, wherein the nonionic surfactant is from about 0.01% to about 1% of the test sample, or about 0.02% to about 0.5% of the test sample, or from about 0.03% to about 0.1% of the test sample, or from about 0.04% to about 0.08% of the test sample, or about 0.05% of the test sample, volume by volume.

48. —The test sample of any one of claims 32-47, wherein the total protein concentration of the test sample is determined with an bicinchoninic acid (BCA) assay, wherein the BCA assay is optionally a micro BCA assay.

49. —The test sample of claim 48, wherein the assay comprises a bicinchoninic acid reagent or a bicinchoninic acid reagent and, optionally, an alkaline tartrate-carbonate buffer and/or a copper sulfate solution.

50. —The test sample of any one of claims 32-49, wherein the test sample comprises 40 mM HEPES, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 0.05% Tween 20.

51. —The test sample of any one of claims 32-50, wherein the pH of the test sample is from about pH 5 to about pH 9, or from about pH 6 to about pH 8, or from about pH 7 to about pH 7.9, or about pH 7.5.

52. —The test sample of any one of claims 32-51, wherein at least one capture reagent is an aptamer, and wherein the at least one aptamer comprises a 5-position modified pyrimidine.

53. —The test sample of claim 52, wherein the 5-position modified pyrimidine comprises a hydrophobic moiety at the 5-position of the pyrimidine.

54. —The test sample of claim 53, wherein the hydrophobic moiety is selected from a naphthyl moiety, a phenyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety.

55. —The test sample of claim 53 or 54, wherein the hydrophobic moiety is covalently linked to the 5-position of the base 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.

56. —The test sample of any one of claims 32-55, wherein the biological sample is a urine sample.

~~57. The test sample of any one of claims 32-55, wherein the biological sample is a serum sample.~~

~~58. The test sample of any one of claims 32-57, wherein gel filtration chromatography was used to perform the buffer exchange on the biological sample in order to generate the buffer-exchanged biological sample.~~

~~59-10.~~ 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 ~~about~~ 70 µg/mL to ~~about less than~~ 100 µg/mL.

~~60-11.~~ The method of claim ~~59-10~~, wherein the at least one protein capture reagent is an aptamer or an antibody.

~~61-12.~~ The method of claim ~~59-10~~ or ~~60-11~~ comprising a plurality of capture reagents, where each capture reagent is an aptamer.

~~62-13.~~ The method of any one of claims ~~59-61-12~~, wherein the total protein concentration of the test sample is ~~about~~ 70 µg/mL to 95 µg/mL; or ~~about~~ 70 µg/mL to 90 µg/mL; or ~~about~~ 70 µg/mL to 85 µg/mL; or ~~about~~ 70 µg/mL to 80 µg/mL; or ~~about~~ 70 µg/mL to 75 µg/mL or ~~about~~ 70 µg/mL.

~~63-14.~~ The method of any one of claims ~~59-62-13~~, wherein the one or more salts are selected from a sodium salt, a potassium salt and a magnesium salt.

~~64-15.~~ The method of any one of claims ~~59-62-13~~, wherein the one or more salts comprise a sodium salt, a potassium salt and a magnesium salt.

~~65.16.~~ The method of claim ~~63-14~~ or ~~6415~~, wherein the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl₂.

~~66.17.~~ The method of claim ~~6516~~, wherein the NaCl in the formulation is at a concentration of from ~~about~~ 10 mM to ~~about~~ 500 mM, or from ~~about~~ 50 mM to ~~about~~ 250 mM, or from ~~about~~ 100 mM to ~~about~~ 200 mM or ~~about~~ 102 mM.

~~67.18.~~ The method of claim ~~65-16~~ or ~~6617~~, wherein the KCl in the formulation is at a concentration of from ~~about~~ 0.5 mM to ~~about~~ 30 mM, or from ~~about~~ 1 mM to ~~about~~ 20 mM, or from ~~about~~ 2 mM to ~~about~~ 15 mM, or from ~~about~~ 4 mM to ~~about~~ 10 mM, or ~~about~~ 5 mM.

~~68.19.~~ The method of any one of claims ~~65-67~~ ~~16-18~~, wherein the MgCl₂ in the formulation is at a concentration of from ~~about~~ 0.5 mM to ~~about~~ 30 mM, or from ~~about~~ 1 mM to ~~about~~ 20 mM, or from ~~about~~ 2 mM to ~~about~~ 15 mM, or from ~~about~~ 4 mM to ~~about~~ 10 mM, or ~~about~~ 5 mM.

~~69.20.~~ The method of any one of claims ~~59-68~~ ~~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.

~~70.21.~~ The method of any one of claims ~~59-69~~ ~~1-20~~, wherein the buffering agent in the formulation is at a concentration of from ~~about~~ 4 mM to ~~about~~ 400 mM, or from ~~about~~ 10 mM to ~~about~~ 300 mM, or from ~~about~~ 20 mM to ~~about~~ 200 mM, or from ~~about~~ 30 mM to ~~about~~ 100 mM, or from 35 mM to ~~about~~ 50 mM or ~~about~~ 40 mM.

~~71.22.~~ The method of any one of claims ~~59-70~~ ~~1-21~~, wherein the chelating agent is selected from EDTA, EGTA, DTPA, BAPTA, DMPS and ALA.

~~72.23.~~ The method of any one of claims ~~59-71~~ ~~1-22~~, wherein the chelating agent in the formulation is at a concentration of ~~about~~ 0.1 mM to ~~about~~ 10 mM, or from ~~about~~ 0.5 mM to ~~about~~ 5 mM, or ~~about~~ 1 mM.

~~73.24.~~ The method of any one of claims ~~59-72~~ ~~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).

~~74.25.~~ The method of any one of claims ~~59-73~~ ~~1-24~~, wherein the nonionic surfactant is from ~~about~~ 0.01% to ~~about~~ 1% of the formulation, or ~~about~~ 0.02% to ~~about~~ 0.5% of the formulation, or

from ~~about~~ 0.03% to ~~about~~ 0.1% of the formulation, or from ~~about~~ 0.04% to ~~about~~ 0.08% of the formulation, or ~~about~~ 0.05% of the formulation.

~~75. The method of any one of claims 59-74, wherein the total protein concentration of the test sample is measured with a bicinchoninic acid (BCA) assay, wherein the BCA assay is optionally a micro-BCA assay.~~

~~76. The method of claim 75, 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.~~

~~77-26.~~ The method of any one of claims ~~59-76~~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.

~~78-27.~~ The method of any one of claims ~~59-76~~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.

~~79-28.~~ The method of any one of claims ~~59-78~~1-26, wherein the pH of the formulation is from ~~about~~ pH 5 to ~~about~~ pH 9, or from ~~about~~ pH 6 to ~~about~~ pH 8, or from ~~about~~ pH 7 to ~~about~~ pH 7.9, or ~~about~~ pH 7.5.

~~80-29.~~ The method of any one of claims ~~59-79~~10-28, wherein the aptamer comprises a 5-position modified pyrimidine.

~~81-30.~~ The method of claim ~~80~~29, wherein the 5-position modified pyrimidine comprises a hydrophobic moiety at the 5-position of the pyrimidine.

~~82-31.~~ The method of claim ~~81~~30, wherein the hydrophobic moiety is selected from a naphthyl moiety, a phenyl moiety, a tyrosyl moiety, an indole moiety and a morpholino moiety.

~~83-32.~~ The method of claim ~~81-30~~ or ~~82~~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.

~~84-33.~~ The method of any one of claims ~~59-83~~10-32, wherein the complex is a non-covalent complex.

~~85-34.~~ The method of any one of claims ~~59-84~~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.

~~86.35.~~ The method of any one of claims ~~59-85~~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.

~~87.~~—~~The method of claim 86, further comprising releasing complex from the first solid support and attaching the complex to a second solid support.~~

~~88.~~—~~The method of claim 87, wherein the complex is attached to the second solid support via the protein.~~

~~89.36.~~ The method of any one of claims ~~59-88~~10-35, further comprising adding a competitor molecule to the test sample and/or adding a competitor molecule and diluting the test sample.

~~90.37.~~ The method of claim ~~89~~36, wherein the competitor molecule is a polyanionic competitor.

~~91.38.~~ The method of claim ~~90~~37, wherein the polyanionic competitor is selected from an oligonucleotide, polydextran, DNA, heparin and dNTPs.

~~92.39.~~ The method of claim ~~91~~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.

~~93.~~—~~The method of claim 91, wherein the polyanionic competitor is an oligonucleotide, and wherein the oligonucleotide comprises one or more 5-position modified pyrimidines.~~

~~94.40.~~ The method of any one of claims ~~59-93~~1-39, wherein the biological sample is urine or wherein the biological sample is serum.

~~95.41.~~ ~~The method of any one of claims 59-93, wherein the biological sample is serum.~~ The method of any one of claims 1-40, wherein the buffer exchange is not performed with ultrafiltration.

~~96.42.~~ The method of any one of claims ~~1-31 or 59-86~~1-41, wherein the buffer exchange is performed using gel filtration chromatography.

~~97.43.~~ The method of any one of claims ~~1-31 or 59-96~~1-42, comprising measuring the protein concentration of the biological sample prior to the performing of the buffer exchange.

~~98.44.~~ The method of claim ~~97~~43, 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.

~~99.45.~~ The method of claim [9844](#), wherein the assay is a BCA assay or a micro BCA assay.

~~100.46.~~ The method of claim [9945](#), 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.

~~101.47.~~ The method of claim [9844](#), wherein the assay is a fluorescence readout assay, optionally wherein the fluorescence readout assay comprises a reagent selected from a merocyanine dye and epicocconone.

~~102.—The method of claim 101, wherein the fluorescence readout assay comprises a reagent selected from a merocyanine dye and epicocconone.~~

~~103.—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 using a formulation comprising a buffering agent, one or more salts, a chelating agent and a nonionic surfactant; and
—determining the total protein concentration of the biological sample or of the test sample; and
—adjusting the total protein concentration of the biological sample or of the test sample, wherein the method does not comprise concentrating the total protein of the biological sample prior to performing the buffer exchange,
—optionally wherein (i) the total protein concentration is adjusted before performing the buffer exchange or (ii) the total protein concentration is adjusted after performing the buffer exchange.~~

~~104.—The method of claim 103, wherein the total protein concentration of the test sample is adjusted after performing the buffer exchange, to from about 2 µg/mL to about 60 µg/mL.~~

~~105.—The method of claim 103 or 104, 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 test sample is about the same or is the same.~~

~~106.—The method of any one of claims 103–105, wherein if the total protein concentration of the biological sample is not in the range of from about 2 µg/mL to about 50 µg/mL; or from about 2 µg/mL to about 45 µg/mL; or from about 4 µg/mL to about 40 µg/mL; or from about 8 µg/mL to about 40 µg/mL; or from about 10 µg/mL to about 40 µg/mL; or from about 10 µg/mL to about 30 µg/mL; or less than 40 µg/mL; or less than 30 µg/mL; or less than 20 µg/mL; or~~

about 15 µg/mL; or from about 10 µg/mL to about 20 µg/mL, the method comprises adjusting the total protein concentration of the biological sample to the range.

107. — The method of any one of claims 103–106, wherein the one or more salts are each independently selected from a sodium salt, a potassium salt and a magnesium salt.

108. — The method of claim 107, wherein the sodium salt is NaCl, the potassium salt is KCl and the magnesium salt is MgCl₂.

109. — The method of any one of claims 103–108, 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, and wherein the buffering agent in the formulation is at a concentration of from about 4 mM to about 400 mM, or from about 10 mM to about 300 mM, or from about 20 mM to about 200 mM, or from about 30 mM to about 100 mM, or from 35 mM to about 50 mM, or about 40 mM.

110. — The method of any one of claims 103–109, wherein the chelating agent is selected from EDTA, EGTA, DTPA, BAPTA, DMPS and ALA, and wherein the chelating agent in the formulation is at a concentration of about 0.1 mM to about 10 mM, or from about 0.5 mM to about 5 mM, or about 1 mM.

111. — The method of any one of claims 103–110, 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), and wherein the nonionic surfactant is from about 0.01% to about 1% of the formulation, or about 0.02% to about 0.5% of the formulation, or from about 0.03% to about 0.1% of the formulation, or from about 0.04% to about 0.08% of the formulation, or about 0.05%, or about 0.1% of the formulation, volume for volume.

112. — The method of any one of claims 103–111, wherein the total protein concentration of the test sample or the biological sample is determined with an assay selected from the group consisting of a fluorescence readout assay, a Bradford assay, a bicinchoninic acid (BCA) assay, a Lowry assay and an ELISA assay.

113. — The method of claim 112, wherein the fluorescence readout assay comprises a reagent selected from a merocyanine dye and epicoconone.

114. —The method of any one of claims 103–113, wherein the formulation comprises 40 mM HEPES, 102 mM NaCl, 5 mM KCl, 5 mM MgCl₂, 1 mM EDTA and 0.05% Tween 20, and wherein the pH of the formulation is from about pH 5 to about pH 9, or from about pH 6 to about pH 8, or from about pH 7 to about pH 7.9, or about pH 7.5.

115. —The method of any one of claims 103–114, wherein the buffer exchange is performed with gel filtration chromatography.

116. —The method of any one of claims 103–115, wherein the biological sample is a urine sample.