Claims

1. A method of determining whether a non-purified sample contains a target protein bound to a ligand of interest comprising the steps of:
a) exposing said non-purified sample to a temperature which is capable of causing or enhancing precipitation of the unbound target protein to a greater extent than it is capable of causing or enhancing precipitation of the target protein bound to said ligand;
b) separating soluble from insoluble protein in the product of step a); and
c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.

2. The method of claim 1 wherein according to step c) the soluble fraction is analysed.

3. The method of claim 1 or claim 2 wherein said non-purified sample is a cell colony, a liquid culture of cells, or a patient or animal sample.

4. The method of any one of claims 1 to 3 wherein said ligand is a protein, DNA or RNA molecule, a cellular metabolite, a drug or another chemical.

5. The method of any one of claims 1 to 4 wherein the temperature is equal to or greater than the initial melting temperature of the target protein.

6. The method of any one of claims 1 to 5 wherein the temperature is greater than 40°C.

7. The method of any one of claims 1 to 6 wherein said non-purified sample is exposed to a series of different temperatures, including a temperature which is equal to or greater than the initial melting temperature of the target.

8. The method of any one of claims 1 to 7 wherein target protein is identified using antibodies.

~~9. The method of any one of claims 1 to 8 wherein the processing step (b) is a step of centrifugation, filtration or affinity separation.~~

~~10. The method of claim 9 wherein target protein in the filtrate from the filtration step is captured on a solid support prior to the analysis step (c).~~

~~11. The method of any preceding claim wherein target protein is added to the sample in order to determine the presence or absence of the ligand of interest in said sample.~~

~~12. The method of any one of claims 1 to 10 wherein a ligand of interest is added to the sample in order to determine the presence or absence of target protein in said sample.~~

~~13. A method for identifying a ligand capable of binding to a target protein comprising the steps of:~~
~~a) exposing a non-purified sample comprising said target protein and a test molecule to a series of different temperatures, including a temperature which is equal to or greater than the initial melting temperature of the target protein;~~
~~b) separating soluble from insoluble protein in the product of step a); and~~
~~c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.~~

~~14. The method of claim 13 wherein a non-purified sample comprising said target protein without added test molecule is also subjected to the method of claim 13 in a control reaction.~~

~~15. A method of determining whether a non-purified sample contains a target protein bound to a ligand of interest, wherein said ligand is not a fusion protein, comprising the steps of:~~
~~a) exposing said non-purified sample to a temperature which is capable of causing or enhancing precipitation of the target protein bound to said ligand to a greater extent than it is capable of causing or enhancing precipitation of the unbound target protein;~~

~~b) separating soluble from insoluble protein in the product of step a); and~~

~~c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.~~

~~16. The method of any one of claims 1 to 7 wherein target protein is identified using antibodies, and wherein step b) is a step of centrifugation, filtration or affinity separation.~~

~~17. The method of any one of claims 1, 13 or 15 wherein said target protein is not detected on the basis of enzymatic activity of a tag, peptide, polypeptide or protein fused thereto.~~

Claims

1. A method of determining whether a non-purified sample contains a target protein bound to a ligand of interest comprising the steps of:

a) exposing said non-purified sample to a temperature which is capable of causing or enhancing precipitation of the unbound target protein to a greater extent than it is capable of causing or enhancing precipitation of the target protein bound to said ligand;

b) separating soluble from insoluble protein in the product of step a); and

c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.

2. The method of claim 1 wherein according to step c) the soluble fraction is analysed.

3. The method of claim 1 or 2 wherein said ligand is a protein, DNA or RNA molecule, a cellular metabolite, a drug or another chemical.

4. The method of any one of claims 1 to 3 wherein the temperature is equal to or greater than the initial melting temperature of the target protein.

5. The method of any one of claims 1 to 4 wherein the temperature is greater than 40°C.

6. The method of any one of claims 1 to 5 wherein said non-purified sample is exposed to a series of different temperatures, including a temperature which is equal to or greater than the initial melting temperature of the target.

7. The method of any one of claims 1 to 6 wherein target protein is identified using antibodies.

8. The method of any one of claims 1 to 7 wherein the processing step (b) is a step of centrifugation, filtration or affinity separation.

9. The method of claim 8 wherein target protein in the filtrate from the filtration step is captured on a solid support prior to the analysis step (c).

10. The method of any preceding claim wherein target protein is added to the sample in order to determine the presence or absence of the ligand of interest in said sample.

11. The method of any one of claims 1 to 9 wherein a ligand of interest is added to the sample in order to determine the presence or absence of target protein in said sample.

12. A method for identifying a ligand capable of binding to a target protein comprising the steps of:
a) exposing a non-purified sample comprising said target protein and a test molecule to a series of different temperatures, including a temperature which is equal to or greater than the initial melting temperature of the target protein;
b) separating soluble from insoluble protein in the product of step a); and
c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.

13. The method of claim 12 wherein a non-purified sample comprising said target protein without added test molecule is also subjected to the method of claim 12 in a control reaction.

14. A method of determining whether a non-purified sample contains a target protein bound to a ligand of interest, wherein said ligand is not a fusion protein, comprising the steps of:
a) exposing said non-purified sample to a temperature which is capable of causing or enhancing precipitation of the target protein bound to said ligand to a greater extent than it is capable of causing or enhancing precipitation of the unbound target protein;
b) separating soluble from insoluble protein in the product of step a); and

c) analysing either or both the soluble and insoluble protein fractions of step b) for the presence of target protein, wherein said target protein is detected by affinity binding a detection moiety or by mass spectrometry.

15. The method of any one of claims 1 to 6 wherein target protein is identified using antibodies, and wherein step b) is a step of centrifugation, filtration or affinity separation.

16. The method of any one of claims 1, 12 or 14 wherein said target protein is not detected on the basis of enzymatic activity of a tag, peptide, polypeptide or protein fused thereto.

17. The method of any one of claims 1 to 16 wherein said non-purified sample is a cell colony, a liquid culture of cells, or a patient or animal sample.

18. The method of claim 17, wherein the patient or animal sample is a sample obtained directly from the patient or animal and/or is a tissue sample, blood, serum, plasma or lymph.

19. The method of any one of claims 1 to 18, wherein the target protein in said non-purified sample is comprised within or on cells.

20. The method of any one of claims 1 to 19, wherein the non-purified is subjected to step a) before a step of lysis is carried out.

21. The method of any one of claims 1 to 20 wherein the non-purified sample is a cell colony, separation step b) is filtration and wherein said cell colony is lifted on a filter and lysis is carried out directly on the colonies on the filter.