CLAIMS

1. An image capillary isoelectric focusing (iCIEF) method for analyzing a change in charge variants of a vascular endothelial growth factor trap (VEGF-Trap) protein comprising:

loading a protein sample onto a separation capillary tube comprising a mixture of at least a carrier ampholyte, methylcellulose, and urea;

applying a first voltage for a first predetermined period of time such that the carrier ampholyte forms a pH gradient within the capillary;

applying a second voltage for a second predetermined period of time to focus the migration of charge variants of the VEGF-Trap protein within the capillary tube such that the overall charge of the variants is neutral; and

detecting and quantifying charge variants of the VEGF-Trap protein comprising:

(i) measuring an absorbance for a plurality of charge variant isoforms;

(ii) segregating the plurality of charge variant isoforms into isolated regions comprising at least a first acid/acidic region (R1), a second neutral region (R2) comprising three principal peaks, and a third base/basic region (R3); and

(iii) determining a percentage of charge variant isoforms falling within each of regions R1, R2 and R3.

2. The method of claim 1, wherein both detecting and quantifying of charge variants are performed.

3. The method of claim 1 or 2, wherein detection of charge variants occurs at a wavelength of 280 nm.

4. The method of any one of claims 1 to 3, wherein the concentration of protein loaded onto the capillary tube is 2.0 mg/ml.

5. The method of any one of claims 1 to 4, wherein the amount of urea in the mixture comprises 1 M to 3 M.

6. The method of any one of claims 1 to 5, wherein the mixture includes 0.35% methylcellulose.

7. The method of any one of claims 1 to 6, wherein the first voltage comprises 1500 V.

8. The method of any one of claims 1 to 7, wherein the first predetermined time is 1 minute.

9. The method of any one of claims 1 to 8, wherein the second voltage comprises 3000 V.

10. The method of any one of claims 1 to 9, wherein the second predetermined time is 7 minutes.

11. The method of any one of claims 1 to 10, wherein the concentration of protein loaded onto the capillary tube is between 1.0 and 8.0 mg/ml, or any intervening range.

12. The method of any one of claims 1 to 11, wherein the amount of urea in the mixture is greater than 0M and less than 8M, or any intervening range.

13. The method of any one of claims 1 to 12, wherein the mixture includes between 0.01% and 0.35% methylcellulose, or any intervening range.

14. The method of any one of claims 1 to 13, wherein the first voltage is between 1 V and 3000 V, or any intervening range.

15. The method of any one of claims 1 to 14, wherein the first predetermined time is between 1 second and 5 minutes, or any intervening range.

16. The method of any one of claims 1 to 15, wherein the second voltage is between 1 V and 6000 V, or any intervening range.

17. The method of any one of claims 1 to 16, wherein the second predetermined time is between 1 minute and 14 minutes, or any intervening range.

18. The method of any one of claims 1 to 17, wherein the mixture includes 0.35% methylcellulose, 2 M urea, and 3% carrier ampholyte having a pI of 3 to 10.

19. The method of any one of claims 1 to 18, wherein the capillary tube includes a fluorocarbon coating.

20. The method of any one of claims 1 to 19, wherein the VEGF-Trap protein comprises the sequence of SEQ ID NO: 1.

21. The method of any one of claims 1 to 20, wherein:

a) the mixture comprises 2M urea, 0.35% methylcellulose, and 3% ampholyte having a pI of 3-10;

b) the first voltage comprises 1500V and the first predetermined time is 1 minute;

c) the second voltage comprises 3000 V and the second predetermined time is 7 minutes; and

d) the VEGF-Trap comprises the sequence of SEQ ID NO: 1.

22. The method of claim 21, wherein the concentration of VEGF-Trap loaded onto the capillary tube is 1.0 mg/mL.