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 of SEQ ID NO:1, the method comprising:

loading a protein sample comprising the VEGF-Trap protein of SEQ ID NO:1 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 charge 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 an acidic region (R1), a neutral region (R2), and a basic region (R3), wherein:

R2 comprises a cluster of three principal peaks that migrate around the neutral isoelectric point (pl) range and are the most prominent charge variant isoforms;

R1 comprises the group of peaks that are relatively acidic compared to the cluster of three principal peaks, and

R3 comprises the group of peaks that are relatively basic compared to the cluster of three principal peaks; 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 the neutral pI range is identified using an independent marker peak.

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:

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, and

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

21. The method of claim 20, wherein the concentration of VEGF-Trap loaded onto the capillary tube is 1.0 mg/mL.