

CLAIMS

1. A method for detecting neurofilament light chain (Nfl) in a biological sample, the method comprising
 - (a) providing a biological sample selected from a blood sample or a CSF sample;
 - (b) enriching for one to a plurality of Nfl isoforms in the biological sample; and
 - (c) detecting one to a plurality of Nfl isoforms enriched in step (b).
2. The method of claim 1,
wherein step (b) comprises
 - (i) contacting the biological sample with a first epitope-binding agent that specifically binds a first population of Nfl isoforms, and isolating the first population of Nfl isoforms thereby producing a sample depleted of the first population of Nfl isoforms;
 - (ii) contacting the sample depleted of the first population of Nfl isoforms with a second epitope-binding agent that specifically binds a second population of Nfl isoforms, and isolating the second population of Nfl isoforms thereby producing a sample depleted of the first and the second populations of Nfl isoforms; and
 - (iii) contacting the sample depleted of the first and second populations of Nfl isoforms with a third epitope-binding agent that specifically binds a third population of Nfl isoforms, and isolating the third population of Nfl isoforms;wherein step (c) comprises detecting one to a plurality of Nfl isoforms in the first population of Nfl isoforms, one to a plurality of Nfl isoforms in the second population of Nfl isoforms, one to a plurality of Nfl isoforms in the third population of Nfl isoforms, or any combination thereof.
3. The method of claim 2, wherein
 - the first epitope-binding agent specifically binds to a first epitope within amino acids 90 to 300 of SEQ ID NO:1, optionally within amino acids 90 to 250 of SEQ ID NO:1;
 - the second epitope-binding agent specifically binds to a second epitope within amino acids 200 to 450 of SEQ ID NO:1 that is downstream of the first epitope; and
 - the third epitope-binding agent specifically binds to a third epitope within amino acids 397 to 543 of SEQ ID NO:1 that is downstream of the second epitope.
4. The method of claim 2 or 3, wherein

the first epitope-binding agent is selected from HJ30.1 or an antigen-binding fragment thereof, HJ30.2 or an antigen-binding fragment thereof, HJ30.13 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.1, HJ30.2 or HJ30.13 binding to full-length recombinant Nfl;

the second epitope-binding agent is selected from HJ30.4 or an antigen-binding fragment thereof, HJ30.7 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.4 or HJ30.7 binding to full-length recombinant Nfl;

the third epitope-binding agent is selected from HJ30.11, an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.11 binding to full-length recombinant Nfl;

or any combination thereof.

5. The method of claim 1, wherein step (b) comprises
 - (i) contacting the biological sample with a first epitope-binding agent that specifically binds a first population of Nfl isoforms, and isolating the first population of Nfl isoforms thereby producing a sample depleted of the first population of Nfl isoforms;
 - (ii) contacting the sample depleted of the first population of Nfl isoforms with a second epitope-binding agent that specifically binds a second population of Nfl isoforms, and isolating the second population of Nfl isoforms;wherein step (c) comprises detecting one to a plurality of Nfl isoforms in the first population of Nfl isoforms, one to a plurality of Nfl isoforms in the second population of Nfl isoforms, or any combination thereof.
6. The method of claim 5, wherein

the first epitope-binding agent specifically binds to a first epitope within amino acids 90 to 450 of SEQ ID NO:1; and/or

the second epitope-binding agent specifically binds to an epitope within amino acids 396 to 543 of SEQ ID NO:1,

optionally wherein the first epitope is within amino acids 250 to 400 of SEQ ID NO:1 and the second epitope is within amino acids 400 to 543 of SEQ ID NO:1.
7. The method of claim 5 or 6, wherein

the first epitope-binding agent is selected from

- (i) HJ30.1 or an antigen-binding fragment thereof, HJ30.2 or an antigen-binding fragment thereof, HJ30.13 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.1, HJ30.2 or HJ30.13 binding to full-length recombinant Nfl, or
- (ii) HJ30.4 or an antigen-binding fragment thereof, HJ30.7 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.4 or HJ30.7 binding to full-length recombinant Nfl;

and/or

the second epitope-binding agent is HJ30.11, an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.11 binding to full-length, recombinant Nfl.

8. The method of claim 1,
 wherein step (b) comprises contacting the biological sample with an epitope-binding agent that specifically binds a first population of Nfl isoforms, and isolating the first population of Nfl isoforms; wherein the epitope-binding agent is selected from the group consisting of:

- (i) an epitope-binding agent that specifically binds to an epitope within amino acids 90 to 250 of SEQ ID NO:1 or amino acids 125 to 250 or SEQ ID NO:1;
- (ii) an epitope-binding agent that specifically binds to an epitope within amino acids 116 to 184 of SEQ ID NO:1;
- (iii) an epitope-binding agent that specifically binds to an epitope within amino acids 250 to 400 of SEQ ID NO:1;
- (iv) an epitope-binding agent that specifically binds to an epitope within amino acids 283 to 338 of SEQ ID NO:1;
- (v) an epitope-binding agent that specifically binds to an epitope within amino acids 400 to 543 of SEQ ID NO:1;
- (vi) an epitope-binding agent that specifically binds to an epitope within amino acids 437 to 543 of SEQ ID NO:1;
- (vii) HJ30.1 or an antigen-binding fragment thereof, HJ30.2 or an antigen-binding fragment thereof, HJ30.13 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.1, HJ30.2 or HJ30.13 binding to full-length, recombinant Nfl

- (viii) HJ30.4 or an antigen-binding fragment thereof, HJ30.7 or an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.4 or HJ30.7 binding to full-length, recombinant Nfl; and
 - (ix) HJ30.11, an antigen-binding fragment thereof, or an epitope-binding agent that competitively inhibits HJ30.11 binding to full-length, recombinant Nfl.
9. The method of claim 1, wherein step (b) comprises enriching for Nfl isoform(s) that are
- (i) about 350 amino acids in length or less, about 300 amino acids in length or less, about 250 amino acids in length or less, about 200 amino acids in length or less, about 150 amino acids in length or less, or about 100 amino acids in length or less;
 - (ii) about 100 amino acids to about 250 amino acids in length; and/or
 - (iii) about 106 amino acids in length or less.
10. The method of any one of claims 1 to 9, wherein step (b) comprises enriching for one to a plurality of Nfl isoforms that have an amino acid sequence comprising
- (i) amino acids 92 to 100 of SEQ ID NO:1, amino acids 101 to 107 of SEQ ID NO:1, amino acids 108 to 116 of SEQ ID NO:1, amino acids 117 to 126 of SEQ ID NO:1, amino acids 137 to 144 of SEQ ID NO:1, amino acids 148 to 157 of SEQ ID NO:1, amino acids 158 to 164 of SEQ ID NO:1, amino acids 165 to 172 of SEQ ID NO:1, amino acids 178 to 185 of SEQ ID NO:1, amino acids 192 to 196 of SEQ ID NO:1, or amino acids 198 to 206 of SEQ ID NO:1;
 - (ii) 283 to 293 of SEQ ID NO:1, amino acids 324 to 331 of SEQ ID NO:1, amino acids 332 to 339 of SEQ ID NO:1, or amino acids 340 to 354 of SEQ ID NO:1;
 - (iii) amino acids 438 to 462 of SEQ ID NO:1 or amino acids 530 to 540 of SEQ ID NO:1; or
 - (iv) amino acids 438 to 462 of SEQ ID NO:1 or amino acids 530 to 540 of SEQ ID NO:1.
11. The method of any one of claims 1 to 10, wherein step (b) enriches for one or more Nfl isoforms comprising an N-terminal truncation, wherein the N-terminal truncation for each Nfl isoform comprises an amino acid sequence independently selected from the group consisting of:
- (1) amino acids 1 to 90 of SEQ ID NO:1;
 - (2) amino acids 1 to 92 of SEQ ID NO:1;

- (3) amino acids 1 to 124 of SEQ ID NO:1;
- (4) amino acids 1 to 135 of SEQ ID NO:1;
- (5) amino acids 1 to 137 of SEQ ID NO:1;
- (6) amino acids 1 to 146 of SEQ ID NO:1;
- (7) amino acids 1 to 162 of SEQ ID NO:1;
- (8) amino acids 1 to 222 of SEQ ID NO:1;
- (9) amino acids 1 to 223 of SEQ ID NO:1;
- (10) amino acids 1 to 234 of SEQ ID NO:1;
- (11) amino acids 1 to 252 of SEQ ID NO:1;
- (12) amino acids 1 to 271 of SEQ ID NO:1;
- (13) amino acids 1 to 281 of SEQ ID NO:1;
- (14) amino acids 1 to 323 of SEQ ID NO:1;
- (15) amino acids 1 to 339 of SEQ ID NO:1;
- (16) amino acids 1 to 359 of SEQ ID NO:1;
- (17) amino acids 1 to 396 of SEQ ID NO:1;
- (18) amino acids 1 to 399 of SEQ ID NO:1;
- (19) amino acids 1 to 420 of SEQ ID NO:1;
- (20) amino acids 1 to 436 of SEQ ID NO: 1;
- (21) amino acids 1 to 437 of SEQ ID NO: 1; and
- (22) amino acids 1 to 528 of SEQ ID NO: 1,

optionally, wherein step (b) enriches for a single isoform.

12. The method of any one of claims 1 to 11, wherein detection in step (c) occurs by mass spectrometry.

13. The method of claim 12, wherein the method further comprises in step (c):

cleaving the enriched Nfl isoforms with a protease and then optionally desalting the resultant cleavage product by solid phase extraction to obtain a sample comprising proteolytic peptides of Nfl; and

performing liquid chromatography-mass spectrometry (LC/MS) of the sample comprising proteolytic peptides of Nfl to detect and optionally quantify the amount of at least one proteolytic peptide of Nfl.

14. The method of claim 13, wherein the at least one proteolytic peptide of Nfl detected and optionally quantified includes

- (1) a peptide with an amino acid sequence chosen from (i) amino acids 165 to 172 of SEQ ID NO:1, (ii) amino acids 198 to 206 of SEQ ID NO:1, (iii) amino acids 324 to 331 of SEQ ID NO:1, (iv) amino acids 438 to 462 of SEQ ID NO:1, or (v) amino acids 530 to 540 of SEQ ID NO:1;
- (2) two or more peptides with an amino acid sequence chosen from (i) amino acids 165 to 172 of SEQ ID NO:1, (ii) amino acids 198 to 206 of SEQ ID NO:1, (iii) amino acids 324 to 331 of SEQ ID NO:1, (iv) amino acids 438 to 462 of SEQ ID NO:1, or (v) amino acids 530 to 540 of SEQ ID NO:1; or
- (3) three or more peptides with an amino acid sequence chosen from (i) amino acids 165 to 172 of SEQ ID NO:1, (ii) amino acids 198 to 206 of SEQ ID NO:1, (iii) amino acids 324 to 331 of SEQ ID NO:1, (iv) amino acids 438 to 462 of SEQ ID NO:1, or (v) amino acids 530 to 540 of SEQ ID NO:1.

15. A method of detecting a biomarker in a sample obtained from a subject, the method comprising detecting neurofilament light chain according to a method of any one of claims 1 to 14, wherein the biological sample is a sample obtained from a subject having or at risk of having neuronal damage and the biomarker is the one to the plurality of Nfl isoforms detected in step (c).

16. The method of any one of claims 1 to 15, further comprising quantifying the one to the plurality of Nfl isoforms detected in step (c).

17. The method of claim 15 or 16, wherein the method comprises quantifying a first and a second population of Nfl isoforms, and optionally wherein the biomarker is a ratio of the two populations.

18. The method of claim 17, wherein

the first population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(i) and the second population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(ii), optionally wherein the population of isoforms of claim 2(i) and claim 2(ii) are produced according to any one of claims 3, 4, or 12-14;

the first population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(i) and the second population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(iii), optionally wherein the population of isoforms of claim 2(i) and claim 2(iii) are produced according to any one of claims 3, 4, or 12-14; or

the first population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(ii) and the second population of Nfl isoforms is the population of Nfl isoforms isolated in claim 2(iii), optionally wherein the population of isoforms of claim 2(ii) and claim 2(iii) are produced according to any one of claims 3, 4, or 12-14.

19. An isolated anti-neurofilament light chain (Nfl) epitope-binding agent selected from
 - (a) HJ30.1, a humanized version of HJ30.1, or an epitope-binding agent that competitively inhibits HJ30.1 binding to full-length recombinant Nfl;
 - (b) HJ30.2, a humanized version of HJ30.2, or an epitope-binding agent that competitively inhibits HJ30.2 binding to full-length recombinant Nfl;
 - (c) HJ30.4, a humanized version of HJ30.4, or an epitope-binding agent that competitively inhibits HJ30.7 binding to full-length recombinant Nfl;
 - (d) HJ30.7, a humanized version of HJ30.7, or an epitope-binding agent that competitively inhibits HJ30.7 binding to full-length recombinant Nfl;
 - (e) HJ30.11, a humanized version of HJ30.11, or an epitope-binding agent that competitively inhibits HJ30.11 binding to full-length recombinant Nfl; or
 - (f) HJ30.13, a humanized version of HJ30.13, or an epitope-binding agent that competitively inhibits HJ30.13 binding to full-length recombinant Nfl.
20. An isolated antibody produced by the hybridoma deposited with the American Type Culture Collection having an ATCC Designation selected from
 - (a) PTA-126966;
 - (b) PTA-126967;
 - (c) PTA-126968;
 - (d) PTA-126969;
 - (e) PTA-126970; or
 - (f) PTA-126971.
21. An isolated anti-neurofilament light chain (Nfl) antibody or antigen-binding fragment thereof, comprising
 - (a) a light chain variable region of HJ30.1 (ATCC # PTA-126966), and/or a heavy chain variable region of HJ30.1 (ATCC # PTA-126966);
 - (b) a light chain variable region of HJ30.2 (ATCC # PTA-126967), and/or a heavy chain variable region of HJ30.2 (ATCC # PTA-126967);

- (c) a light chain variable region of HJ30.4 (ATCC # PTA-126968), and/or a heavy chain variable region of HJ30.4 (ATCC # PTA-126968);
 - (d) a light chain variable region of HJ30.7 (ATCC # PTA-126969), and/or a heavy chain variable region of HJ30.7 (ATCC # PTA-126969);
 - (e) a light chain variable region of HJ30.11 (ATCC # PTA-126970), and/or a heavy chain variable region of HJ30.11 (ATCC # PTA-126970); or
 - (f) a light chain variable region of HJ30.13 (ATCC # PTA-126971), and/or a heavy chain variable region of HJ30.13 (ATCC # PTA-126971).
22. An isolated anti-neurofilament light chain (Nfl) antibody or antigen-binding fragment thereof, comprising
- (a) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.1 (ATCC # PTA-126966), and/or a heavy chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.1 (ATCC # PTA-126966);
 - (b) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.2 (ATCC # PTA-126967), and/or a heavy chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.2 (ATCC # PTA-126967);
 - (c) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.4 (ATCC # PTA-126968), and/or a heavy chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.4 (ATCC # PTA-126968);
 - (d) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.7 (ATCC # PTA-126969), and/or a heavy chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.7 (ATCC # PTA-126969);
 - (e) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.11 (ATCC # PTA-126970), and/or a heavy chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.11 (ATCC # PTA-126970); or
 - (f) a light chain variable region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity to the VL of HJ30.13 (ATCC # PTA-126971), and/or a heavy chain variable

region with 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the VH of HJ30.13 (ATCC # PTA-126971).

23. Use of the isolated anti-Nfl epitope-binding agent of claim 19, the isolated antibody of claim 20, or the isolated anti-Nfl antibody or antigen-binding fragment thereof of claim 21 or 22, for detecting and/or quantifying Nfl in a biological sample, optionally wherein the biological sample is a blood sample or a cerebrospinal fluid sample, and further optionally wherein the epitope-binding agent, or antibody or antigen-binding fragment thereof is attached to a detectable label.