

CLAIMS

1. A method for measuring the amount of dystrophin protein in a biological sample comprising:
 - (i) contacting the sample with a protease, forming a protein digest comprising dystrophin peptides;
 - (ii) applying the protein digest to an affinity matrix comprising antibodies, or antigen binding fragments thereof, specific for at least one dystrophin peptide;
 - (iii) eluting dystrophin peptides bound to the affinity matrix, forming a dystrophin peptide-enriched sample; and
 - (iv) performing liquid-chromatography-mass spectrometry (LC/MS) on said dystrophin peptide-enriched sample.
2. The method of claim 1, further comprising lysing the biological sample in a lysis buffer.
3. The method of claim 2, wherein the lysis buffer is a RIPA buffer comprising a detergent.
4. The method of any one of claims 1 to 3, further comprising precipitating proteins in the biological sample with an organic solvent.
5. The method of claim 4, wherein the organic solvent is selected from the group consisting of acetic acid, acetone, acetonitrile, dimethylformamide, dimethyl sulfoxide, dioxane, ethanol, isopropanol, methanol, 1-propanol, and tetrahydrofuran.
6. The method of any one of claims 1 to 5, further comprising solubilizing the protein precipitate with a solution comprising trypsin and a chaotropic agent.
7. The method of any one of claims 1 to 6, wherein said protease is trypsin.
8. The method of any one of claims 1 to 7, further comprising reduction and alkylation of the solubilized protein protein precipitate.
9. The method of any one of claims 1 to 8, further comprising quantifying the amount of dystrophin protein in the sample relative to a reference.

10. The method of any one of claims 1 to 9, wherein said dystrophin protein comprises the amino acid sequence of any one of SEQ ID NO:242, SEQ ID NO:243, SEQ ID NO:244, or SEQ ID NO:245.
11. The method of any one of claims 1 to 10, wherein said dystrophin peptide-enriched sample comprises a peptide consisting of the amino acid sequence of any one of SEQ ID NO:1 to SEQ ID NO:241.
12. The method of any one of claims 1 to 11, wherein said dystrophin peptide-enriched sample comprises a peptide consisting of the amino acid sequence of any one of SEQ ID NO:235 to SEQ ID NO:241.
13. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in the amino acid sequence each of full length wild type human dystrophin protein and that of an engineered dystrophin protein.
14. The method of claim 13, wherein said peptide amino acid sequence consists of SLEGSDDAVLLQR (SEQ ID NO:179) or LLQVAVEDR (SEQ ID NO:200).
15. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in the amino acid sequence of full length wild type human dystrophin protein but not that of an engineered dystrophin protein.
16. The method of claim 15, wherein said peptide amino acid sequence consists of ILADLEEENR (SEQ ID NO:224).
17. The method of claim 11 or 12, wherein said peptide amino acid sequence is present contiguously in an engineered dystrophin protein but not that of full length wild type human dystrophin protein.
18. The method of claim 17, wherein said peptide amino acid sequence consists of LEMPSSLMLEVPTHR (SEQ ID NO:236).
19. The method of any one of claims 1 to 18, wherein said biological sample is a sample of skeletal muscle obtained from a human diagnosed with Duchenne's muscular dystrophy (DMD).
20. The method of claim 19, wherein said human with DMD is treated with a gene therapy vector expressing an engineered dystrophin protein comprising the amino acid sequence of SEQ ID NO:243.