



TENDON HOMOGENIZATION USING THE MINILYS

Proteomics Research in a Major Medical Center, Minnesota

/ CONTEXT

Proteomics involves the analysis and identification of protein structure and function. Through the Proteomics Core, investigators have access to a broad range of proteomics services to help them answer scientific questions. For this particular research project on tendon healing, the Minilys homogenizer was used to homogenize minced or intact tendon for protein extraction, followed by mass spectrometry analysis to identify unique peptides.

/ MATERIALS

- Minilys homogenizer
- Lysing kits: CKMix50-R 2mL (KT03961-1-013.2) and CK68-R 2mL (KT03961-1-014.2)
- Samples: Minced or intact Achilles tendon from dog (wet weight 36-44 mg)
- Buffer: 300 μ l of 2% SDS/50mM Tris, pH 8

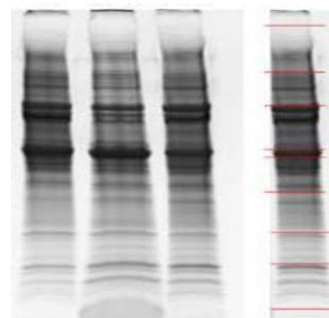
/ PROTOCOL

- Minilys parameters: 5000 rpm, 3 cycles of 60 seconds, rest at room temperature for 3 minutes in between each cycle
- After the last cycle, the lysates were transferred to microcentrifuge tubes. Each lysing tube was washed with another 50 μ l of buffer, and the supernatant added to the appropriate tube
- Protein concentration was measured, and 20 μ g of each lysate was run on a SDS-PAGE gel followed by Coomassie Blue staining
- Sample lane #3 was cut into 8 sections, and each section was digested with trypsin, followed by mass spectrometry analysis using Nano LC-MS/MS on a Thermo Q-Exactive Hybrid Quadrupole Orbitrap Mass Spectrometer (8 MS runs, respectively)

/ RESULTS

Lysing tube	Tendon	Wet weight	Total protein yield	ug protein/mg wet weight
CK68-R 2mL	Intact	44 mg	361 μ g	8.19 μ g
CKMix50-R 2mL	Intact	40 mg	245 μ g	6.12 μ g
CKMix50-R 2mL	Minced	36 mg	478 μ g	13.28 μ g

Table 1. Protein extraction efficiency from dog tendon was compared between the CK68 and CKMix50 beads after homogenization using the Minilys.



Lane 1: Intact tendon, homogenized with CK68-R 2mL

Lanes 2 and 3: Intact (2) and minced (3) tendon, homogenized with CKMix50-R 2mL

Figure 1. Protein band patterns are compared using Coomassie Blue staining after SDS-PAGE electrophoresis.

The highest protein yields were observed by mincing the tendon first, prior to homogenization with the CKMix50-R 2mL tubes (Table 1). However, the protein band profiles of all three treatment groups were similar (Figure 1). Lane #3 was cut into different sections, trypsin-digested and analyzed using mass spectrometry, which led to the identification of 152 total unique proteins (a minimum of ≥ 2 peptides from each protein had to be detected for a confident ID). This included contaminating serum proteins like albumin, but the majority identified were unique collagen and extracellular matrix proteins.

/ CONCLUSION

The Minilys is an efficient homogenizer that can process up to three samples in 2mL or 0.5 mL tubes simultaneously. When coupled with the appropriate lysing matrix, the Minilys can effectively process tough tissues such as tendon for DNA, RNA or protein extraction.

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【Minilys を用いたイヌ アキレス腱からのタンパク質抽出】

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