APPLICATION NOTE

Multi-tissue protein extraction across different tube formats and reagent types using 5 mm Omni Tips.

Authors

Aamna Aijaz
Olivia Koopman
Caleb Proctor

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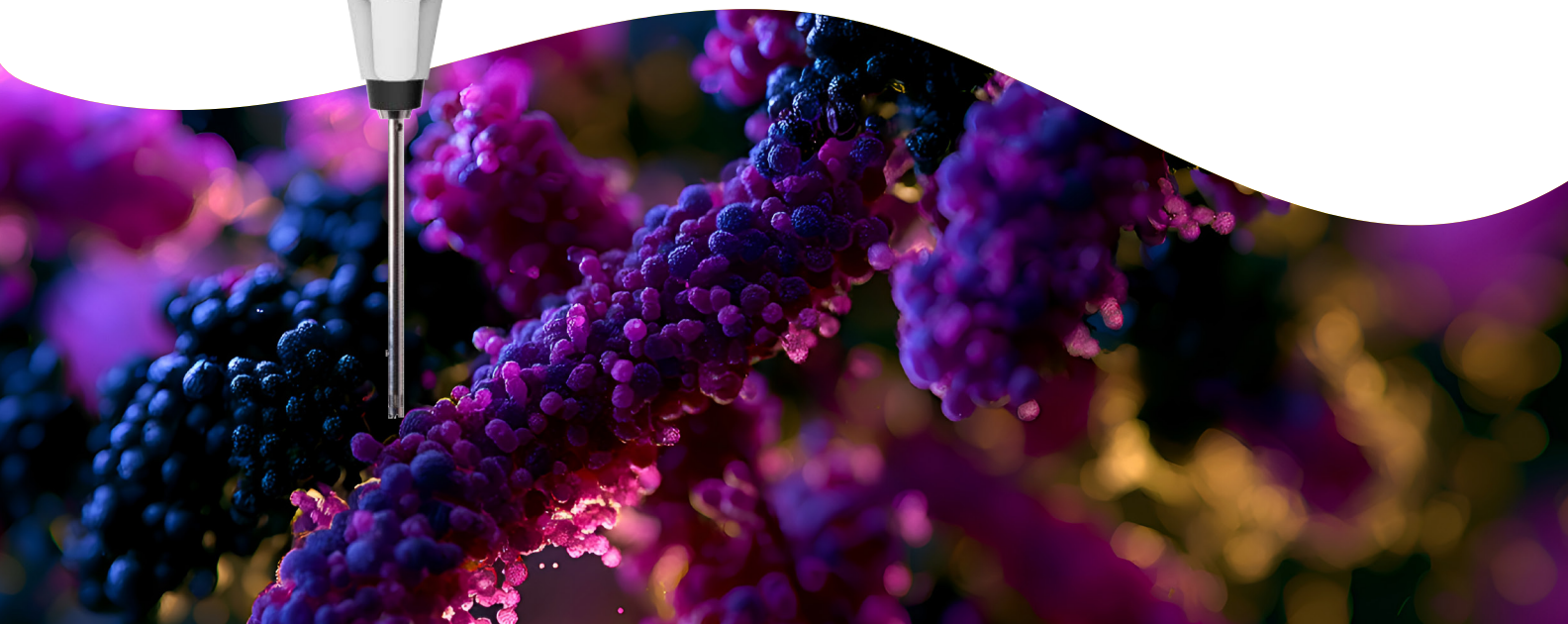
Omni THQ digital tissue homogenizer



Summary

Protein extraction and analysis workflows are a fundamental component for biological research, providing insight into cellular function and biochemical responses. The reliability of such insights, however, is contingent on the experimental conditions governing the accuracy and reproducibility of the analysis[1]. Variability can be introduced at any stage of the experiment, yet it is most commonly introduced during sample preparation particularly for solid tissue samples[2]. These inconsistencies in homogenization and lysis efficiency propagate downstream, ultimately compromising the accuracy and reproducibility of experimental outcomes, making effective homogenization a critical determinant of data quality and experimental success.

Among the most influential factors governing sample preparation efficiency is the choice of lysis strategy. Researchers commonly rely on chemical lysis buffers, such as radioimmunoprecipitation assay (RIPA) buffer, for protein extractions[3]. These aqueous buffers commonly contain ionic and non-ionic detergents designed to disrupt cellular membranes while solubilizing proteins. Phosphate-buffered saline (PBS) is also utilized in applications that require a milder extraction to reduce detergent exposure. While chemical buffers and enzymatic digestion remain widely used, mechanical disruption in sample preparation is a more efficient alternative.

We have previously demonstrated that mechanical disruption using the Omni Bead Ruptor Elite™ bead mill homogenizer effectively lyses cells and releases intracellular contents, reducing the dependency on lysis buffer for cell disruptions, allowing researchers to be more flexible in selecting a buffer based on downstream application requirements rather than lysis efficiency alone[4]. Handheld rotor-stator homogenizers represent another mechanical disruption tool, particularly well-suited for heat-sensitive analytes and low-throughput laboratories, offering a cost-effective solution for tissue homogenization, suspension preparation, and cell disruption. Additionally, the rotor stator technology utilized in handheld homogenizers can be readily automated. This supports researchers to confidently conduct small-scale research using handheld homogenizers, then immediately translate that research into an environment that can accommodate more samples than a single user can reliably work with at any given time. This setup collectively allows for validation of workflows from the bench to rapidly deployed in broader research environments.

In this application note, we evaluate the use of the Omni THQ digital tissue homogenizer paired with 5 mm Omni Tips™ for homogenizing tissue samples for protein extraction and downstream analysis. The Soft Tissue 5 mm Omni Tip offers flexibility by supporting homogenization of various sample sizes in smaller tubes with reduced working volumes, while maintaining the efficiency and accuracy of protein recovery. The homogenization performance of the Soft Tissue 5 mm Omni Tip was evaluated by processing *Mus musculus* livers, kidneys, spleens, and skeletal muscle. Various tissue sample sizes were processed using multiple tube sizes and varying volumes of either PBS or RIPA lysis buffer to assess homogenization performance under different conditions. Following tissue disruption and cellular lysis, total protein concentration was quantified using a bicinchoninic acid (BCA) assay, a colorimetric assay utilized for its sensitivity, compatibility with various detergents and reproducibility in protein determination. The results were analyzed to evaluate protein extraction yield, and consistency of cellular lysis across all conditions and ultimately demonstrate effectiveness of the Soft Tissue 5 mm Omni Tip for high-quality tissue homogenization and protein extraction in low-volume applications.

Materials and methods

Equipment

- Omni THQ digital tissue homogenizer (part number # 12-500)
- Soft Tissue Omni Tip Plastic Homogenizing Probes, 5 mm (500/pk) (part number # 35550)

Procedure

Sample acquisition:

Mus musculus livers, kidneys, spleens, and skeletal muscles were excised and frozen at -80°C. Organs were thawed on the day of experimentation.

5 mL tube homogenization:

For homogenization in 5 mL tubes, 1 mL of PBS or RIPA lysis buffer was added to each 5 mL round-bottom tube containing 10, 50, or 100 mg tissue samples (± 1 mg) of liver (n=3 per sample size), kidney (n=1 per sample size), muscle (n=3 per sample size), or spleen (n=1 per sample size). Samples were homogenized using 5 mm Omni Tips on the Omni THQ homogenizer at 20,000 rpm for the processing times specified in Table 1. Following homogenization, samples were centrifuged at 4,000 rpm for 5 minutes. Cleared lysates were diluted 1:10 with PBS to bring protein concentrations within the linear range of the BCA assay.

2 mL tube homogenization:

For homogenization in 2 mL tubes, 200 µL of PBS or RIPA lysis buffer was added to 2 mL microcentrifuge tubes containing 10, 25, or 50 mg tissue samples (± 1 mg) of liver (n=3 per sample size) or muscle (n=3 per sample size). Samples were homogenized using 5 mm Omni Tips on the Omni THQ homogenizer at 20,000 rpm for the processing times specified in Table 2. Following homogenization, samples were centrifuged at 10,000 rpm for 5 minutes. Liver lysates were diluted 1:20 and muscle lysates were diluted 1:10 with PBS prior to analysis to for the protein concentrations to fall within the linear range of the BCA assay.

1.5 mL tube homogenization:

For homogenization in 1.5 mL tubes, 100 µL of PBS or RIPA lysis buffer was added to each tube containing 10, 25, or 50 mg tissue samples (± 1 mg) of liver (n=3 per sample size). Samples were homogenized using 5 mm Omni Tips on the Omni THQ homogenizer at 20,000 rpm for the processing times specified in Table 3. Following homogenization, samples were centrifuged at 10,000 rpm for 5 minutes. Liver lysates were diluted 1:100 with PBS prior to analysis to bring protein concentrations within the linear range of the BCA assay.

BCA protein assay:

For protein quantification, 25 µL of diluted homogenate was loaded in triplicate into a 96-well microplate, followed by the addition of 200 µL BCA working reagent. Protein standards were prepared using bovine serum albumin (BSA) diluted in the corresponding sample buffer matrix to generate a standard curve ranging from 20–2,000 µg/mL. Separate standards were prepared in PBS and 0.1× RIPA buffer diluted in PBS to match sample conditions. Plates were incubated at room temperature for 30 minutes, and absorbance was measured at 562 nm using a microplate reader.

Statistical analysis:

All data collected from the BCA assay were exported in Microsoft® Excel, and all statistical analyses were performed using Dotmatics' GraphPad® Prism version (10.2.3). Since the different tube formats initially received varying buffer volumes and were subsequently diluted to bring the concentrations within the assay's detectable range, the results from the 2 mL tubes were normalized to allow for comparison with the results from the 5 mL and 1.5 mL tubes. Specifically, the liver sample results were divided by 2.5, while the muscle sample results obtained from the 2 mL tubes were divided by 5. The 5 mL and 1.5 mL tubes were at the same dilution level at the end and did not require normalization. All graphical figures and table values were created in Dotmatics' GraphPad®.

Table 1: Processing Parameters for 5 mL tubes with Omni THQ and 5 mm Omni Tips at 20,000 rpm in 1 mL buffer.

Organ	Size (mg)	Processing time (s)
Liver/Kidney/Spleen	100	60
	50	45
	10	30
Muscle	100	120
	50	105
	10	30

Table 2: Processing Parameters for 2 mL tubes with Omni THQ and 5 mm Omni Tips at 20,000 rpm in 200 µL buffer.

Organ	Size (mg)	Processing time (s)
Liver	50	30
	25	30
	10	45
Muscle	50	60
	25	60
	10	30

Table 3: Processing Parameters for 1.5 mL tubes with Omni THQ and 5 mm Omni Tips at 20,000 rpm in 100 µL buffer.

Organ	Size (mg)	Processing time (s)
Liver	50	60
	25	60
	10	60

Results

The Soft Tissue 5 mm Omni Tips demonstrated effective homogenization across all tissue types and processing conditions evaluated. To assess performance across a range of workflows, homogenization was conducted in three different tube formats with varying working volumes, and protein yield was quantified by BCA assay.

Protein extraction in PBS across tube formats

Homogenization of all four tissue types in 5 mL tubes with 1 mL PBS demonstrated a strong linear relationship between tissue input mass and protein yield, with $R^2 > 0.99$ across liver, kidney, spleen, and skeletal muscle (Figure 1a, Table 4a). Reproducibility across biological replicates was high for liver at all sample sizes (<5%) tested while only the larger sample sizes (50 and 100 mg, <5%) of muscle samples returned consistent results. The 10 mg muscle samples exhibited greater variability, likely reflecting the inherent heterogeneity of small skeletal muscle samples. Skeletal muscle yielded lower protein concentrations relative to other tissue types at equivalent sample sizes.

Homogenization of liver and skeletal muscle in 2 mL tubes with 200 µL PBS similarly produced sample size-dependent protein recovery across 10, 25, and 50 mg sample sizes, with strong linearity observed for both tissue types (Figure 1a, Table 4a). Liver replicates demonstrated excellent reproducibility across all sample sizes, while 10 mg muscle samples again showed higher variability compared to the 25 and 50 mg groups.

Liver tissue homogenized in 1.5 mL tubes with 100 µL PBS maintained strong linearity and consistent protein recovery across the tested sample range (Figure 1a, Table 4a). Variability was higher at the 10 mg input but decreased substantially at larger sample sizes.

Protein extraction in RIPA buffer across tube formats

Homogenization of all four tissue types in 5 mL tubes with 1 mL RIPA buffer yielded sample size-dependent protein recovery across all sample sizes, with strong linearity observed for liver, kidney, spleen, and skeletal muscle (Figure 1b, Table 4b). Liver and muscle replicates were highly reproducible at 50 and 100 mg sample sizes, with RSDs of ≤6%. As observed with PBS, the 10 mg muscle samples showed the greatest variability across biological replicates (RSD = 45%), likely attributable to the structural heterogeneity of small skeletal muscle inputs. Skeletal muscle consistently yielded lower protein concentrations relative to the other tissue types at equivalent sample sizes, in line with PBS findings.

Liver and skeletal muscle homogenized in 2 mL tubes with 200 µL RIPA buffer demonstrated consistent, sample size-dependent protein recovery across 10, 25, and 50 mg sample sizes (Figure 1b, Table 4b). Liver reproducibility was strong across all sample sizes (RSD ≤ 10%), while muscle variability was highest at 10 mg (RSD = 9%) and improved at larger inputs, with the 50 mg group achieving an RSD of ≤1%.

Liver tissue processed in 1.5 mL tubes with 100 µL RIPA buffer-maintained sample size-dependent protein recovery across the tested sample range (Figure 1b, Table 4b). Variability was highest at the 10 mg input (RSD = 16%) but decreased markedly at 25 and 50 mg (RSDs of 4% and 2%, respectively), consistent with trends observed across both buffer conditions and tube formats.

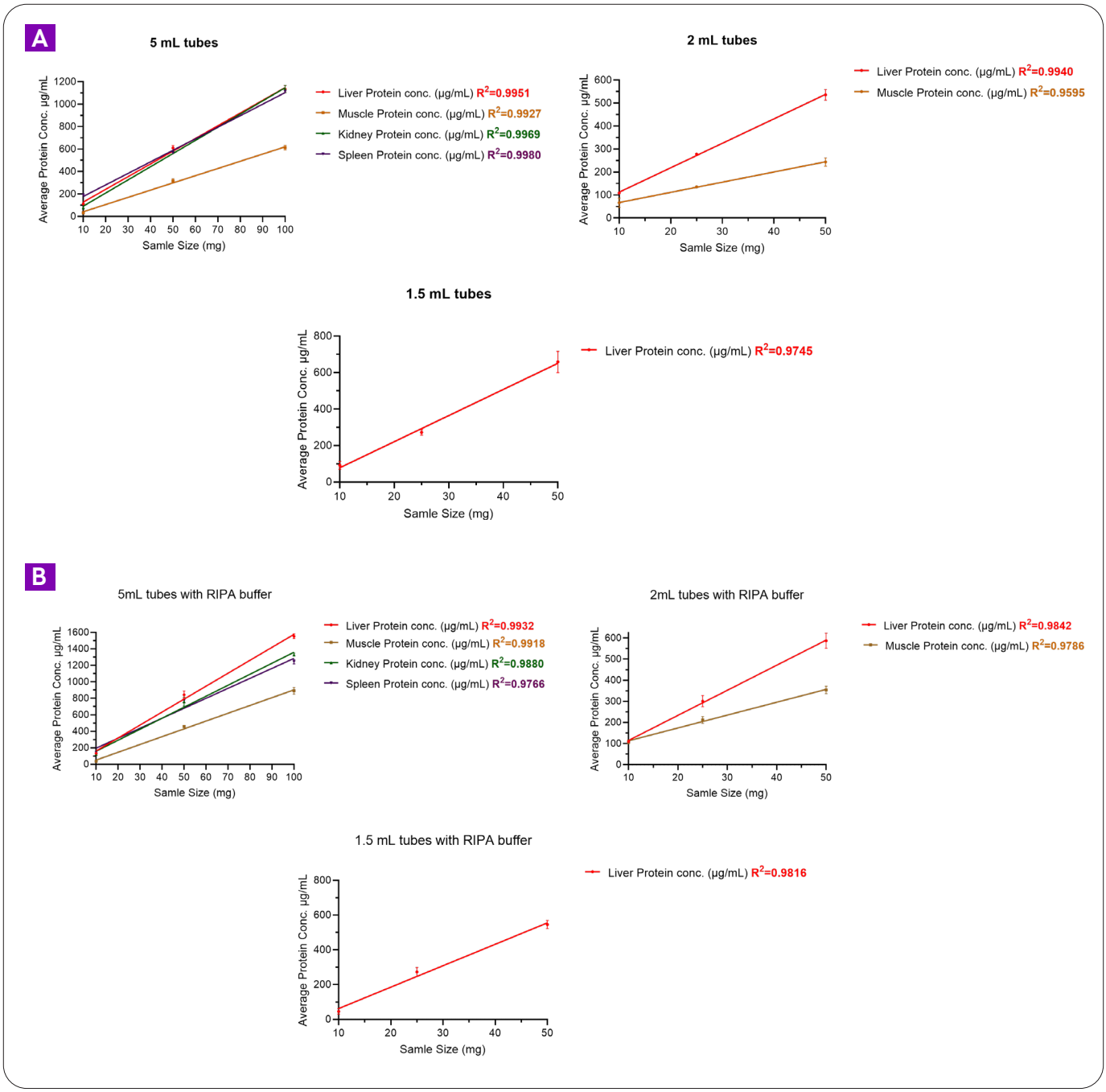


Figure 1: BCA-derived protein concentration as a function of tissue input mass (10–100 mg) for lysates prepared in (a) PBS or (b) RIPA lysis buffer. Samples were processed in 5 mL tubes containing 1 mL buffer, 2 mL tubes containing 200 µL buffer, or 1.5 mL tubes containing 100 µL buffer. Data are shown for liver, muscle, kidney, and spleen tissues and demonstrate strong linearity across the tested concentration range, as indicated by the R^2 values. Reported protein concentrations were normalized by correcting for the respective dilution factors applied during sample preparation.

Table 4: BCA-derived protein concentrations of lysates prepared from liver, muscle, kidney, and spleen tissues using tissue inputs ranging from 10–100 mg and processed in 5 mL, 2 mL, or 1.5 mL tubes with varying (a) PBS and (b) RIPA lysis buffer volumes. The mean from triplicate reads represents the average of the three technical replicate absorbance measurements, and the corresponding standard deviation (SD) reflects variability among these technical replicates. The overall mean was calculated from all measurements across replicates for each tissue type, while the overall SD represents variability across all measurements for one sample size. For liver and muscle tissues, the overall statistics were derived from nine total plate measurements obtained from three samples analyzed in triplicate, whereas kidney and spleen statistics were derived from three plate measurements obtained from a single sample analyzed in triplicate.

Tissue type	Tube size (mL)	Tissue size (mg)	Number of samples (n)	Mean protein concentration from triplicate reads (µg/mL)	SD of triplicate reads (µg/mL)	Overall mean protein concentration (µg/mL)	Overall SD (µg/mL)	Overall RSD (%)
Liver	5	10	3	117.3	2.6	112.6	4.2	4%
				109.4	4.8			
				111.1	2.2			
		50	3	603.1	6.3	609.8	22.2	4%
				634.6	20.4			
				591.7	4.8			
		100	3	1130.1	2.2	1134.7	16.1	1%
				1121.5	49.5			
				1152.6	25.6			
Muscle	5	10	3	36	1.8	32.49	9.8	30%
				21.4	1.8			
				40.1	2.7			
		50	3	304.6	5.3	306.3	5.4	2%
				301.9	1.8			
				312.3	1			
		100	3	613.2	5.1	612.1	23	4%
				634.6	7.4			
				588.6	7.5			
Kidney	5	10	1	*to reduce redundancy, means and SD were carried over to next columns since only one sample was tested.		77.6	2.7	4%
		50	1			585.7	6	1%
		100	1			1134.9	32.8	3%
Spleen	5	10	1			191.1	1.1	1%
		50	1			566.1	3.1	1%
		100	1			1112.7	7.8	1%
Liver	2	10	3	110.9	2.3	108.6	2.3	2%
				106.2	0.5			
				108.8	0.7			
		25	3	275.2	1.8	277.8	3.1	1%
				276.7	1.1			
				281.3	5.8			
		50	3	517.2	2.3	535.6	26	5%
				524.3	10.7			
				565.3	6.7			
Muscle	2	10	3	88.5	23.1	66.2	19.5	30%
				57.6	1.3			
				52.4	0.1			
		25	3	135.8	1.9	135.6	0.2	0.1%
				135.8	1.4			
				135.4	2.7			
		50	3	245.5	22.6	243.9	5.6	2%
				248.5	13.7			
				237.7	23.1			
Liver	1.5	10	3	72.3	4.7	91.5	25.5	28%
				81.7	4.6			
				120.5	3.4			
		25	3	253.5	6	272.2	17.1	6%
				276.1	6.3			
				287	4			
		50	3	735.1	10.8	658.7	66.6	10%
				628	15.5			
				612.9	8.6			

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Tissue type	Tube size (mL)	Tissue size (mg)	Number of samples (n)	Mean protein concentration from triplicate reads (µg/mL)	SD of triplicate reads (µg/mL)	Overall mean protein concentration (µg/mL)	Overall SD (µg/mL)	Overall RSD (%)
Liver	5	10	3	137.1	17.9	133.6	4.4	3%
				128.7	8.1			
				134.9	5.2			
		50	3	870.6	9.5	843.3	50.9	6%
				784.6	22.5			
				874.6	5.7			
		100	3	1557.8	35.2	1552.7	16.2	1%
				1565.8	28.8			
				1534.6	18.1			
Muscle	5	10	3	51.9	2.2	36.3	16.2	45%
				19.5	5			
				37.5	7.9			
		50	3	449.5	3.9	458.4	9.6	2%
				457.2	18.5			
				468.6	11.7			
		100	3	930.5	22.3	890.7	42.9	5%
				896.3	15.4			
				845.2	14.4			
Kidney	5	10	1	*to reduce redundancy, means and SD were carried over to next columns since only one sample was tested.		123.2	9	7%
		50	1			759.2	54.7	7%
		100	1			1330.7	17.3	1%
Spleen	5	10	1			144.1	8.1	6%
		50	1			774.3	23.2	3%
		100	1			1242.8	26.3	2%
Liver	2	10	3	107.8	1.2	110	4.3	4%
				107.3	2.3			
				114.9	3.6			
		25	3	273.5	2.1	301.4	30	10%
				297.5	4.2			
				333.1	2.7			
		50	3	589.2	3.2	587.3	40.9	7%
				627.3	3.6			
				545.5	4.4			
Muscle	2	10	3	103.3	0.5	108.6	10.3	9%
				120.5	6.4			
				102.1	3.4			
		25	3	200.1	5.3	212	17.7	8%
				203.6	4.1			
				232.3	7.7			
		50	3	353.1	18.7	354.2	2.2	1%
				352.8	25.2			
				356.7	13.9			
Liver	1.5	10	3	49.6	18	45.9	7.5	16%
				50.8	13.7			
				37.3	18.7			
		25	3	285.7	23.9	273.3	11.6	4%
				271.5	27.8			
				262.7	28.5			
		50	3	560.3	23.8	545.6	12.9	2%
				540.5	25.1			
				536.1	21.5			

Conclusions

The 5 mm Soft Tissue Omni Tip in combination with the Omni THQ demonstrated effective homogenization resulting in reproducible protein extraction across a range of tissue types, sample sizes and buffer conditions. Homogenization of liver, kidney, spleen and skeletal muscle in both PBS and RIPA buffer yielded sample size-dependent protein recovery across all three tube formats evaluated. Strong linearity was observed across tissue types and tube formats in both buffer conditions, supporting the reliability of the 5 mm Omni Tip for consistent homogenization and protein recovery regardless of working volume. The 5 mm Omni Tip's compatibility with both detergent-based RIPA buffer and mild PBS extraction demonstrates its suitability for diverse workflows confirming that buffers do not hinder the sample homogenization.

The ability to process samples in reduced working volumes as low as 100 µL in 1.5 mL tubes, while maintaining reproducible protein yields, also highlights the 5 mm Omni Tip's utility in low-input and volume-limited workflows. Together, these findings establish the 5 mm Omni Tip as a flexible, disposable and efficient solution for tissue homogenization, particularly well-suited for low-throughput laboratories and applications. These findings also support a pathway to sample prep automation on the LH96 automated homogenizer workstation for laboratories seeking to scale and streamline the weighing, diluting, homogenization, and reformatting steps of their sample preparation workflows.

References

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