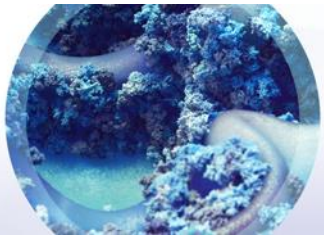

Pushing performance for low-input proteomics applications using Enhanced Sensitivity Mode on the ZenoTOF 8600 system

Patrick Pribil and Jason Causon

SCIEX, Canada

This technical note demonstrates how Enhanced Sensitivity Mode (ESM) further extends the ZenoTOF 8600 system's performance for low-input quantitative proteomics by refining instrument parameters and detector tuning to improve detection and amplification of low-level ions. At sample loadings as low as 100 pg, ESM increases the number of detectable and quantifiable protein groups and precursors by as much as 40%, while improving quantitative reproducibility as much as 34%. By extending quantitative depth and confidence from picogram-level inputs, ESM helps proteomics researchers generate more reproducible measurements from limited material and unlocks new performance for high-sensitivity applications such as single-cell proteomics.

Key features of ZT Scan DIA 3.0 with Enhanced Sensitivity Mode on the ZenoTOF 8600 system

- **Unlock up to 40% more quantifiable biology from just 100 pg of sample:** With Enhanced Sensitivity Mode, the ZenoTOF 8600 system delivers up to 40% more quantifiable protein groups and precursors from low-input commercial human lysate digests, enabling deeper proteome coverage when every ion matters.
- **See more. Quantify with greater confidence:** Enhanced Sensitivity Mode improves spectral quality while increasing peptide peak areas and quantitative precision, providing greater confidence in peptide identification and quantitation—especially for low-abundance signals that can define biological discovery.

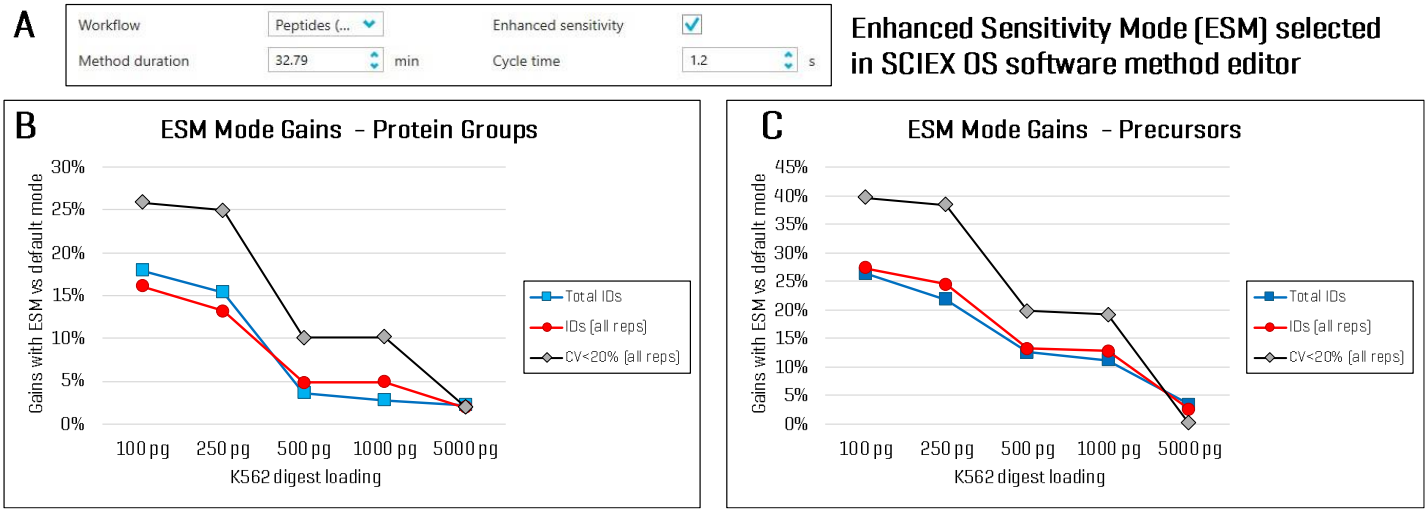

Figure 1. Enhanced Sensitivity Mode (ESM) on the ZenoTOF 8600 system. (A) Enabling ESM in the SCIEX OS software ZT Scan DIA method editor. The performance gains for protein groups (A) and precursors (B) with ESM on various sample loadings of K562 digest, acquired using Whisper Zoom 40 SPD on the Evosep Eno system combined with ZT Scan DIA 3.0 on the ZenoTOF 8600 system. Relative gains in total IDs, features identified in all 3 replicates, and quantified features (CV<20%) with ESM (relative to default mode) are indicated at each loading.

Introduction

For low-input and single-cell proteomics, biological insight is often limited not by sample complexity alone, but by whether low-abundance peptide ions can be detected, confidently assigned, and reproducibly quantified across injections. ESM on the ZenoTOF 8600 system is designed to address this challenge by refining detector tuning and instrument parameters to improve detection and amplification of low-level ions, with the greatest impact expected for picogram-to-low-nanogram sample loads. By combining ESM's enhanced low-level ion detection with ZT Scan DIA 3.0's Zeno trap–enabled MS/MS and scanning quadrupole isolation, the platform improves sensitivity, precursor selectivity, and MS/MS data quality to deliver deeper, more reproducible quantitative proteomics from limited material while maintaining DIA's broad sampling advantages. This technical note evaluates performance using a commercial Human lysate digest and a hybrid Human/Yeast/E.coli proteome mixture. The results demonstrate increased numbers of detectable and quantifiable protein groups and precursors, improved quantitative reproducibility, and greater confidence in low-abundance peptide identification and quantitation.

Methods

Sample preparation: Human K562 and Yeast lysate tryptic digests were purchased from Promega. E.coli lysate tryptic digests were purchased from Waters. Lysate digest dilutions were prepared in buffer containing 0.1% formic acid/0.01% N-dodecyl β-D-maltoside detergent in water. For the Human/Yeast/E.coli (HYE) mixtures, lysates were mixed in 3 different percentage weight ratios as previously described (4,5). All samples were loaded onto Evotips (Evosep, Denmark) at the indicated amounts according to Evotip preparation instructions provided by Evosep.

Chromatography: All separations were performed using the Evosep Eno system (Evosep, Denmark) using the Whisper Zoom 40 SPD method. An IonOpticks Aurora Elite XS C18 nanoflow column (15 cm x 0.075 mm) was used, heated to 55 °C. All samples were analyzed in triplicate.

Mass spectrometry: All samples were analyzed using the ZenoTOF 8600 system, with the horizontal nanoflow probe. Ion source and ZT Scan DIA method parameters are described in Table 1. ZT Scan DIA methods had "Enhanced sensitivity" either checked (ESM) or unchecked (default mode) (see Figure 1A).

Table 1: ZenoTOF 8600 system parameters.

Parameter	Value
Method duration	68 min
Curtain gas	40
CAD gas	7
Nano cell temperature	250 °C
Nano gas 1	10 psi
Ion source gas 2	30 psi
Source heaters	150 °C
Nano spray voltage	2,100 V
Total cycle time	1.2 sec
TOF MS m/z range	350-1500 Da
TOF MS accumulation time	50 msec
MS/MS m/z range	140-1750 Da
MS/MS accumulation time	16.6 msec
Zeno mode	On
Dynamic collision energy	On (+2)
ZT Scan DIA Q1 isolation range	370-1025 Da
ZT Scan DIA Q1 width	9.8 Da
ZT Scan DIA Q1 scan speed	590 Da/sec
Total MS/MS scan time	1.125 sec

Data processing: All data were processed using PEAKS Studio software version 13.5. Protein identification was performed using a combined spectral library and FASTA database search strategy. For the K562 lysate digest dataset, searches were conducted using a previously generated K562/HeLa gas-phase fractionation spectral library (6) together with a FASTA database containing canonical Human protein sequences downloaded from UniProt (https://www.uniprot.org). For the HYE hybrid proteome dataset, searches were performed using an HYE gas-phase fractionation spectral library (6) together with a FASTA database containing canonical Human, Yeast, and E. coli protein sequences downloaded from UniProt (https://www.uniprot.org). For all searches, carbamidomethylation of cysteine (C) was specified as a fixed modification, while deamidation (N/Q) and oxidation (M) were included as variable modifications. Match Between Runs (MBR) was enabled for all analyses.
For the K562 dilution series, the 3 replicate injections corresponding to each sample loading and acquisition mode

(ESM or default mode) were searched together. For the HYE label-free quantitation (LFQ) study, all 9 data files corresponding to the 3 HYE samples (3 replicates per sample) acquired using a given mode (ESM or default mode) were searched together. To preserve the expected abundance relationships in the HYE mixtures, LFQ normalization was disabled during data processing. The numbers of identified protein groups and identified precursors were obtained from the exported Protein and Feature Vector LFQ results, respectively.

Enhanced Sensitivity Mode drives progressively larger gains as sample amounts decrease

To evaluate the impact of ESM on low-input proteomics performance, a dilution series of K562 digest ranging from 5 ng to 100 pg—approximating single-cell-level sample loads—was analyzed on the ZenoTOF 8600 system using ZT Scan DIA 3.0 with a 9.8 Da Q1 window width. To maximize sensitivity, separations were performed using the Whisper Zoom 40 SPD method on the Evosep Eno system. Protein groups and precursors identified and quantified across the dilution series, including those consistently detected in all 3 replicates and quantified with CV <20%, are shown in Figures 2 and 3. A summary of the performance gains achieved with ESM is presented in Figure 1. Across the dilution series, ESM delivered clear improvements in proteome depth and quantitative performance, with the largest benefits observed at the lowest sample loads where sensitivity is most critical. As sample input decreased, the gains became progressively more pronounced, culminating in increases of up to 26% in quantified protein groups and up to 40% in quantified precursors at the lowest loading levels. These results demonstrate the ability of ESM to extend the quantitative reach of the ZenoTOF 8600 system into the picogram regime, enabling deeper and more reproducible characterization of sample-limited proteomes.

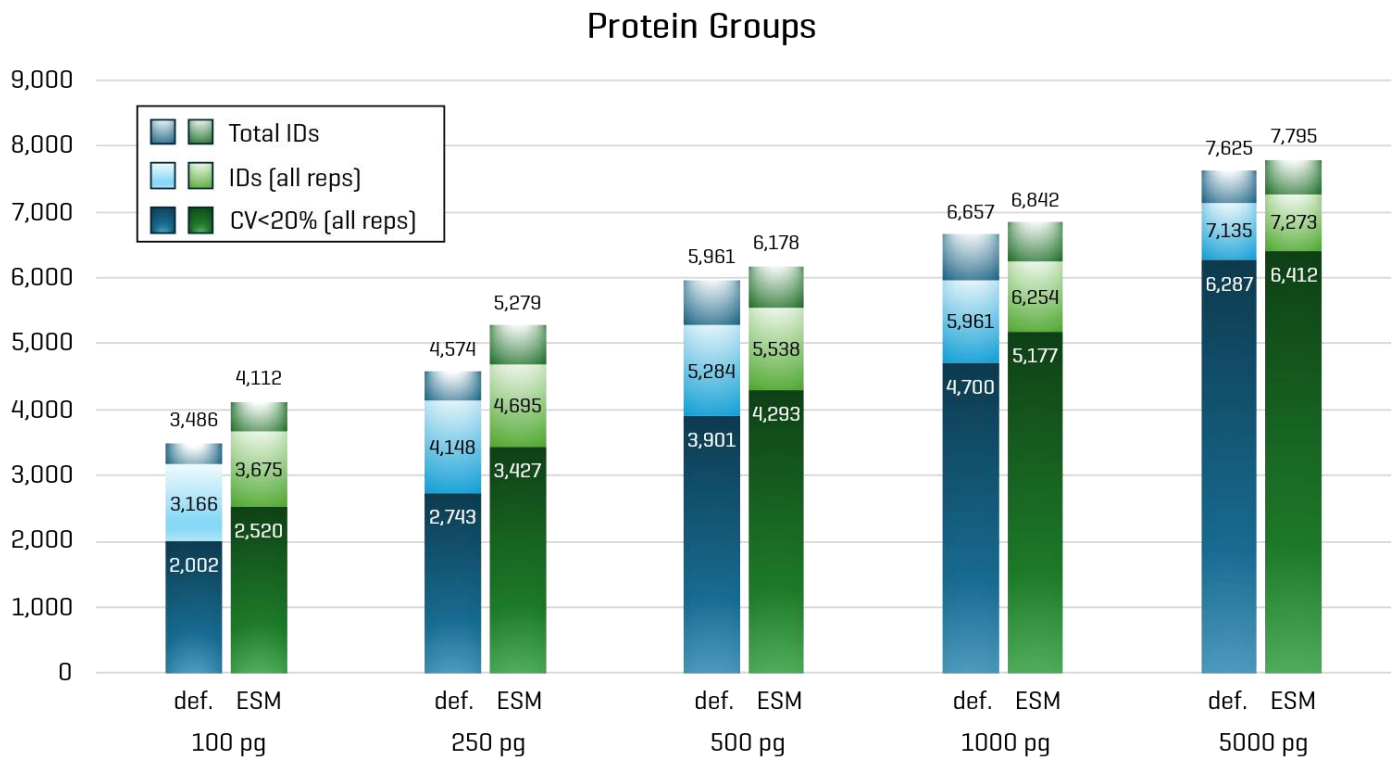

Figure 2. Protein groups identified and quantified in K562 digest. The indicated sample loadings of K562 digest were analyzed using Whisper Zoom 40 SPD on the Evosep Eno system combined with ZT Scan DIA 3.0 on the ZenoTOF 8600 system, acquired in either ESM or default (def.) modes. Data were processed using PEAKS Studio software version 13.5. Samples were analyzed in triplicate for each loading. The total number of protein groups identified, those identified in all 3 replicates, and those quantified in all 3 replicates with CV<20% are indicated.

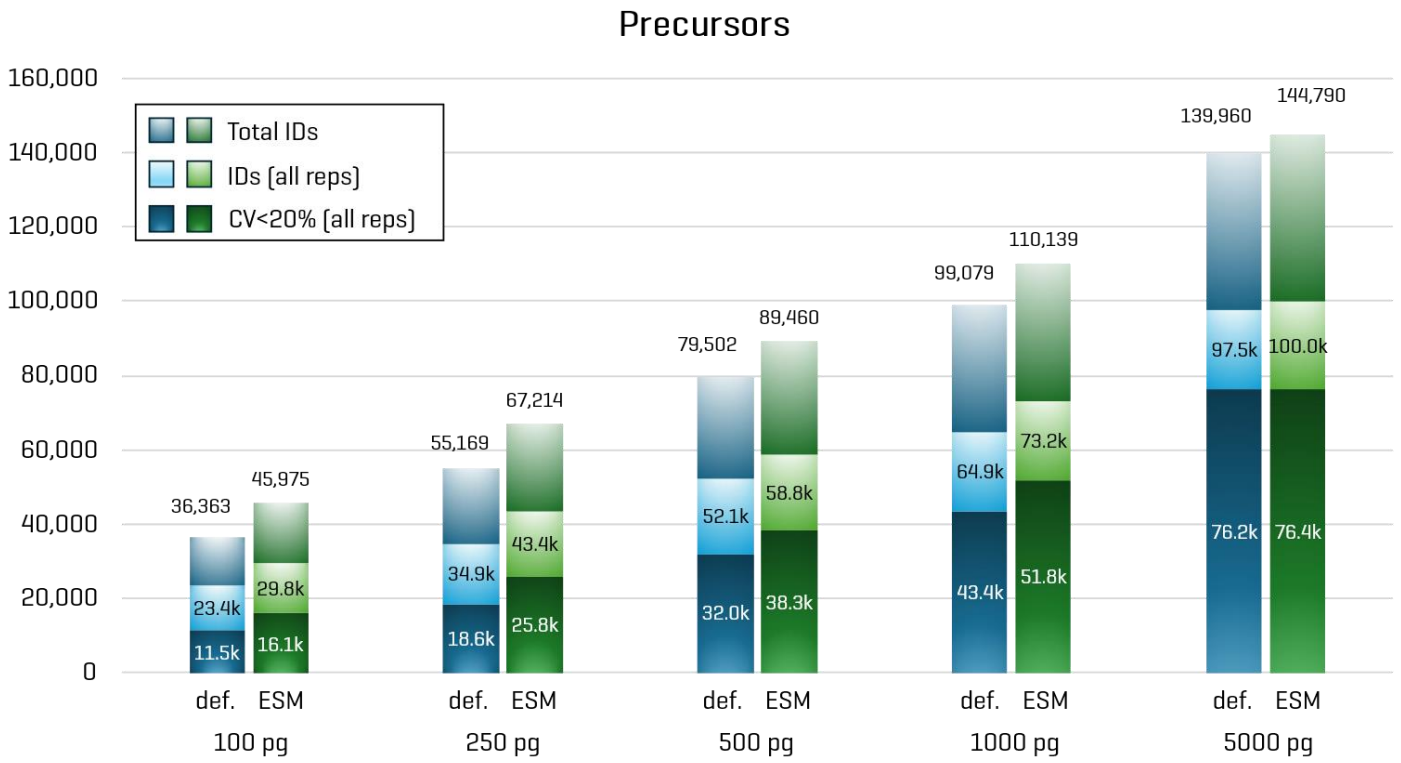

Figure 3. Precursors identified and quantified in K562 digest. The indicated sample loadings of K562 digest were analyzed using Whisper Zoom 40 SPD on the Evosep Eno system combined with ZT Scan DIA 3.0 on the ZenoTOF 8600 system, acquired in either ESM or default (def.) modes. Data were processed using PEAKS Studio software version 13.5. Samples were analyzed in triplicate for each loading. The total number of precursors identified, those identified in all 3 replicates, and those quantified in all 3 replicates with CV<20% are indicated.

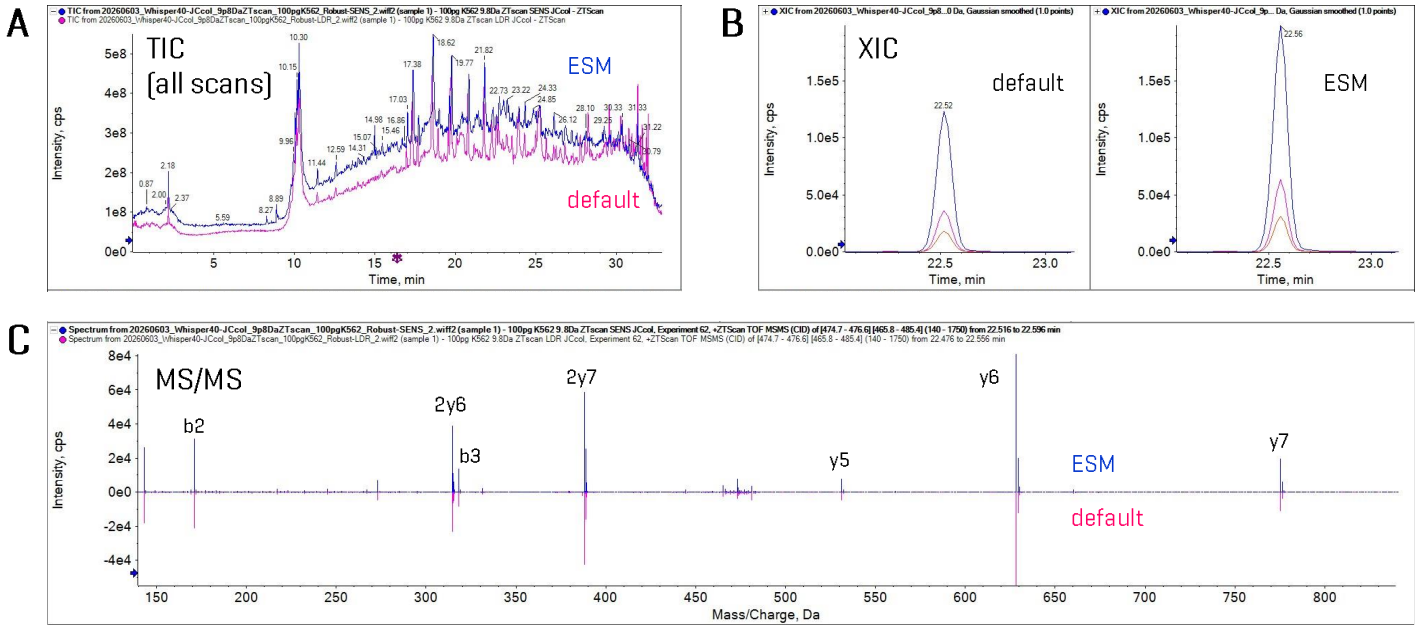

Figure 4. Signal intensity gains using ESM at 100 picogram sample loading of K562 digest. Samples were analyzed using Whisper Zoom 40 SPD on the Evosep Eno system combined with ZT Scan DIA 3.0 on the ZenoTOF 8600 system. (A) Comparison of the total ion chromatograms (TICs) between ESM and default modes. (B) Extracted ion chromatograms (XICs) for the y6 (m/z 628.378), y7 (m/z 775.446) and y5 (m/z 531.325) fragment ions from the ZT Scan DIA MS/MS data for peptide AVFPSIVGR (2+ charge state precursor) from POCG38|POTEI_HUMAN protein. (C) Comparison of MS/MS signal intensity and quality for this peptide. A noticeable increase in TIC signal, XIC signal/peak area, and MS/MS fragment ion intensity is observed with ESM versus default mode.

Enhanced low-level ion detection translates directly into stronger signals and improved quantitation

The improved identification and quantitation observed with ESM reflect enhanced detection and amplification of low-intensity ion signals, enabling stronger peptide- and fragment-level responses. This effect is illustrated in Figure 4 using the peptide AVFPSIVGR from the POCG38|POTEI_HUMAN protein at a sample loading of 100 pg. Total ion chromatograms (TICs, Figure 4A), extracted ion chromatograms (XICs) for 3 representative fragment ions (Figure 4B), and the corresponding MS/MS spectra (Figure 4C) all show substantially stronger signal responses with ESM compared with default mode.

The magnitude of these improvements is summarized in Figure 5, where both XIC peak areas and fragment ion peak heights show average gains of 63% with ESM. By boosting signal intensity at the peptide and fragment-ion level, ESM increases confidence in peptide detection, identification, and quantitation, providing a powerful advantage for low-input and high-sensitivity proteomics workflows.

Fragment Ion	Peak Height (default)	Peak Height (ESM)	Gains Peak Height	XIC Peak Area (default)	XIC Peak Area (ESM)	Gains XIC Peak Area
628.378 (y6)	5.45E+04	8.08E+04	48.3%	6.52E+05	1.04E+06	59.1%
775.446 (y7)	1.12E+04	1.96E+04	75.0%	1.85E+05	3.08E+05	66.8%
531.325 (y5)	4.84E+03	8.04E+03	66.1%	9.53E+04	1.57E+05	65.2%
AVERAGE			**63.1%**			**63.7%**

Figure 5. Gains in peak height/area using ESM. The gains in fragment ion peak height and XIC peak area are summarized for peptide AVFPSIVGR (2+ charge state precursor) from POCG38|POTEI_HUMAN protein from K562 digest, analyzed at a sample loading of 100 pg with either ESM or default modes (see Figure 4C).

Enhanced Sensitivity Mode strengthens quantitative confidence in low-input proteomics

To evaluate the impact of ESM on LFQ performance, 3 HYE hybrid proteome mixtures were analyzed at a sample loading of 250 pg (Figure 6A). Samples were separated on the Evosep Eno system with the Whisper Zoom 40 SPD method and analyzed on the ZenoTOF 8600 system using ZT Scan DIA 3.0 with a 9.8 Da Q1 window width. Data were processed in PEAKS Studio version 13.5, and pairwise comparisons were performed (Sample A vs. Sample B and Sample C vs. Sample B) to assess both the number of precursors quantified across all replicates and the deviation from expected abundance ratios for each species represented in the HYE mixture.

The results, summarized in Figure 6B, demonstrate that ESM delivers measurable improvements in LFQ performance across all comparisons and species. Most notably, the number of precursors quantified in every replicate—eliminating missing values—is increased by approximately 20% with ESM relative to default mode. At the same time, the deviation from expected abundance ratios is reduced in virtually every comparison, indicating improved quantitative accuracy. These gains highlight the ability of ESM to recover and confidently quantify additional low-level signals while maintaining robust quantitative fidelity.

Beyond increasing quantitative coverage and accuracy, ESM also enhances analytical precision. Figure 7 summarizes precursor-level CV values across replicates for each sample and species. In every comparison, ESM produces lower CV values than default mode, with improvements of up to 34%, demonstrating substantially tighter quantitative reproducibility. Taken together, these results show that ESM delivers a powerful combination of deeper quantitative coverage, improved accuracy, and enhanced precision for low-input proteomics workflows. By increasing the number of confidently quantified precursors while simultaneously reducing quantitative variability, ESM enables higher-confidence biological interpretation from sample-limited analyses and further extends the performance of the ZenoTOF 8600 system for next-generation applications, including low-input and single-cell proteomics.

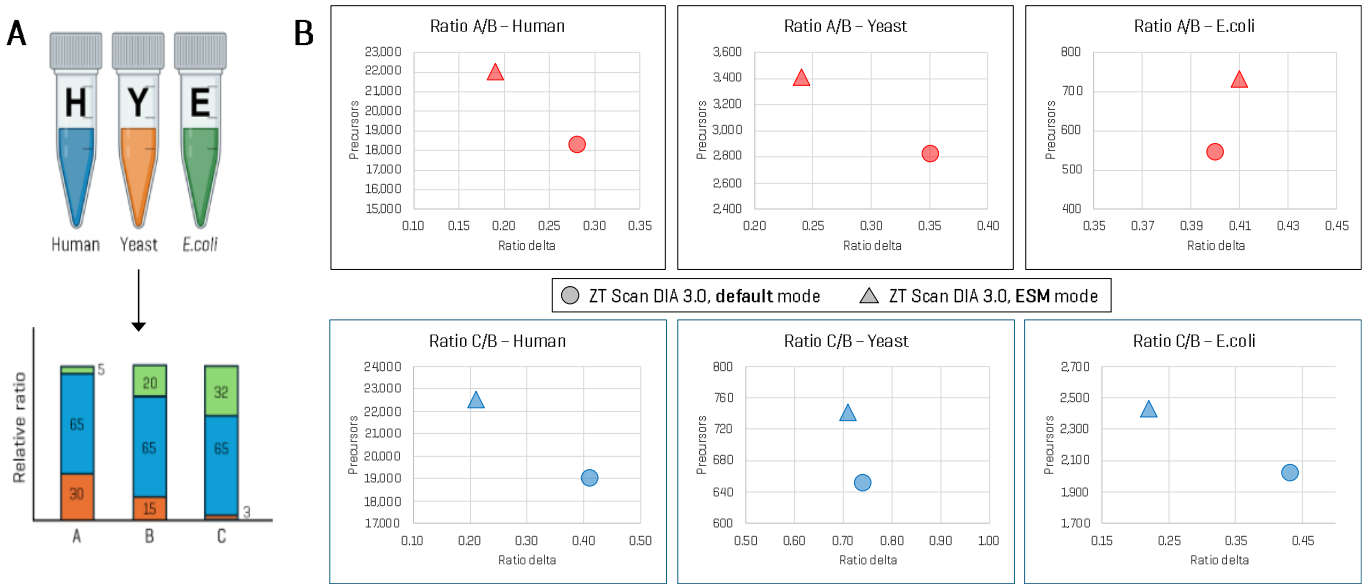

Figure 6. Improvements in precursor identifications and LFQ accuracy using ESM. (A) 250 pg loadings of hybrid proteome mixtures (HYE) were analyzed using Whisper Zoom 40 SPD on the Evosep Eno system combined with ZT Scan DIA 3.0 on the ZenoTOF 8600 system, in either ESM or default modes. For each HYE mixture A, B or C, samples were analyzed in triplicate, and data were processed using PEAKS Studio software version 13.5. (B) Pairwise comparisons of the HYE mixtures were done to determine the number of precursors quantified across all replicates and the deviation in peak area ratios (Ratio delta) from the expected ratios for each pairwise comparison (for each species). Using ESM, the number of quantified precursors was significantly improved for each species. Additionally, the Ratio delta improved in virtually every case, indicating higher LFQ accuracy with ESM than with default mode.

Median Precursor CVs		Human		Yeast		E.coli	
		default	ESM	default	ESM	default	ESM
A vs B	A	19.0	13.2	17.2	10.1	21.9	15.5
	B	13.3	12.6	15.0	13.9	7.7	7.5
C vs B	C	14.7	13.3	17.7	15.3	11.4	10.8
	B	13.7	12.8	7.7	6.7	14.7	13.4

Figure 7. ESM improves LFQ precision. For the analysis of HYE hybrid proteome mixtures in Figure 6, the quantitative precision (i.e. coefficients of variation, or CVs) was determined for precursors quantified in each sample and species in each pairwise comparison. In all cases, ESM yielded lower CV values (i.e. improved quantitative precision) compared to default mode.

Conclusions

- **Push deeper into the proteome at ultra-low sample loads:** Using ESM on the ZenoTOF 8600 system, researchers can achieve up to 40% more quantifiable protein groups and precursors from commercial human digests at sample loadings as low as 100 picograms, extending proteome depth where sensitivity is most critical.
- **Capture more of the ions that matter:** ESM significantly enhances the detection and amplification of low-level ion signals, delivering up to 63% increases in peptide peak height and peak area, enabling stronger signals, improved data quality, and greater confidence in low-abundance measurements.
- **Elevate quantitative performance for next-generation low-input proteomics:** At sample loadings representative of single-cell workflows, ESM increases the number of quantifiable protein groups and precursors while improving label-free quantitation (LFQ) accuracy and precision, empowering more confident biological interpretation from limited material.

References

1. Heymann, T., et al. (2026). Scanning DIA on the ZenoTOF 8600 system enables ultra-sensitive and quantitative proteomics from single cells to post-translational modifications in a compact platform. bioRxiv, https://doi.org/10.64898/2026.03.12.711261.

2. ZT Scan DIA 3.0: A data-independent acquisition (DIA) method with high-resolution precursor selection improves protein identification and quantitation for proteomics research. SCIEX technical note, MKT- 38093-A.

3. Narrow-window scanning DIA for unbiased label-free quantitation: Improved protein group ratio accuracy with ZT Scan DIA 3.0 on the ZenoTOF 8600 system. SCIEX technical note, MKT-38229-A.

4. Accurate label-free quantitative (LFQ) proteomics benchmarking using data-independent acquisition (DIA) on the ZenoTOF 8600 system. SCIEX technical note, MKT-38149-A.

5. Van Puyvelde, B., et al. (2026). LFQ Benchmark Dataset – Generation Beta: Assessing Modern Proteomics Instruments and Acquisition Workflows with High-Throughput LC Gradients. bioRxiv, https://doi.org/10.64898/2026.01.29.702266.

6. Unlocking deeper data-independent acquisition (DIA) performance with empirical spectral libraries using ZT Scan DIA 3.0 on the ZenoTOF 8600 system. SCIEX technical note, MKT-39246-A.

Headquarters
250 Forest Street| Marlborough, MA 01752 USA
Phone 508-383-7700
sciex.com

International Sales
For our office locations please call the division headquarters or refer to our website at
sciex.com/offices