

MIRAM GAGome Kit for quantification of urine CS disaccharides (0S CS, 4S CS, 6S CS)

UPLC-MS/MS Analysis of Chondroitin Sulfate Disaccharides 0S CS, 4S CS and 6S CS for Biomedical Research

APPLICATION BENEFITS

Complete. A complete RUO Kit solution for quantitative analysis of 0S CS, 4S CS and 6S CS in urine, including all key reagents, calibrators, quality controls, analyte-specific internal standards, consumables and Directions for Use. Designed for UHPLC-MS/MS using the Waters Xevo TQ-S Micro.

Quality controlled and standardized.

Integrated calibrators, quality controls and internal standards support consistent quantitative results across samples and studies.

Comprehensive. The three CS disaccharides are measured within one workflow.

Analytically validated. A kit and well-characterized workflow that is developed and analytically validated specifically for CS disaccharide quantification in human urine.

Urinary CS profiling. 0S CS, 4S CS and 6S CS together represent >90% of measurable CS disaccharides in human urine. Their quantitative analysis provides a broad coverage of urinary CS composition and major sulfonation patterns, enabling research into biological variation and changes in urinary CS profiles. Additional CS disaccharides, including 2S CS, 2S4S CS, 2S6S CS, 4S6S CS and 2S4S6S CS are detectable under the specified chromatographic conditions. These additional analytes are not supported by kit-provided calibrators, internal standards or quality controls and are therefore intended for qualitative detection only.

Keywords: MIRAM GAGome Kit, Glycosaminoglycans, Chondroitin Sulfate (CS) disaccharides 0S CS, 4S CS, 6S CS, Waters™ Xevo TQ-S Micro

INTRODUCTION

The MIRAM GAGome Kit for the quantification of urine 0S CS, 4S CS and 6S CS disaccharides is a RUO Kit for the quantitative assessment of three glycosaminoglycan-derived chondroitin sulfate (CS) disaccharides in human urine. The concentrations of non-sulfated chondroitin sulfate, 4-sulfated chondroitin sulfate, and 6-sulfated chondroitin sulfate glycosaminoglycan-derived disaccharides in urine should be quantified using Waters™ Xevo TQ-S Micro platform.

MIRAM GAGome Kit includes reagents and sample preparation components for manual processing to digest chondroitin sulfate glycosaminoglycans (GAGs). The resulting chondroitin sulfate disaccharides are separated by ultraperformance liquid chromatography, and the concentrations of three chondroitin sulfate disaccharides are then detected and quantified by tandem mass spectrometry, namely:

- CS disaccharide with no sulfation (0S CS)
- CS disaccharide with sulfation on the hydroxyls of the 4th position of the N-acetyl-galactosamine (4S CS)
- CS disaccharide with sulfation on the hydroxyls of the 6th position of the N-acetyl-galactosamine (6S CS).

The MIRAM GAGome Kit is applicable to the concentrations of 0S CS, 4S CS and 6S CS disaccharides within their Analytical Measuring Intervals (AMI). The measurements are analytically valid between the respective lower limits of measuring interval (LLMI) and upper limit of measuring interval (ULMI) of the Analytical Measuring Intervals (AMIs):

- 0S CS 1.0 to 25.0 µg/mL
- 4S CS 1.0 to 10.0 µg/mL
- 6S CS 0.7 to 4.0 µg/mL



MIRAM GAGome Kit (FRUOU-CS) Application Note

MATERIALS PROVIDED

Each MIRAM GAGome Kit contains reagents sufficient for one 96-well plate. The plate can include up to 61 urine samples. The rest of the wells shall be filled with Blanks, Calibrators, Quality Control samples, and a Wash solution for the UPLC-MS/MS.

MATERIALS NOT PROVIDED

LC-MS/MS instrument; Lab equipment such as pipettes, vortex, centrifuge, ThermoMixer® (ThermoTop® cover); Consumables such as tubes, bottles, reservoirs; Chemicals for Mobile Phases; Columns for LC-MS/MS analysis.

SAMPLE PREPARATION

Urine samples are thawed at room temperature (if frozen) and then centrifuged at $3000 \times g$ and 9°C for 10 minutes. Box 3 reagents are thawed at room temperature for 40 minutes immediately before processing. Blanks, Calibrators, Quality Controls, Internal Standards, Solvent 1, Solvent 2, and Solvent 3 are vortexed right before use. Blanks, Calibrators, Quality Controls and urine samples are added to a 96-well plate according to the Directions for Use plate layout, followed by Solvent 1, Internal Standards, and Reagent A. The plate is incubated in a ThermoMixer® at 37°C and 600 rpm for 4 hours, followed by addition of Reagent B resuspended in Solvent 2 and Reagent C resuspended in ultrapure water. The plate is then incubated at 40°C and 600 rpm for 16 hours. The following day, Solvent 3 is added and the plate is centrifuged at $2000 \times g$ and 9°C for 10 minutes before being transferred to the UPLC-MS/MS autosampler.

For Directions for Use, please refer to the relevant section of the downloading information provided with the Kit.

MIRAM GAGome Kit

Each MIRAM GAGome Kit contains reagents sufficient for one 96-well plate. The plate can include up to 61 urine samples. The rest of the wells shall be filled with Blanks, Calibrators, Quality Control samples, and a Wash solution for the UPLC-MS/MS.

The MIRAM GAGome Kit reagents are delivered in three individual subunits: MIRAM GAGome Kit Boxes 1-3:

- Boxes 1 and 2 contain all reagents that shall be stored at room temperature (18°C to 25°C). Reagent C is packaged separately in Box 2 because of its hazardous properties (flammable, toxic).
- Box 3 contains all reagents that shall be stored at freezer temperature (-24°C to -18°C).

Box 1 (storage at 18°C to 25°C)

Component	Quantity	Appearance	Content	Preparation prior to use
Reagent B	1 Vial	Dry powder	0.046 g	To be reconstituted with 2.7 mL of Solvent 2
Solvent 2	1 Bottle	Liquid	3.00 mL	Ready for use
Solvent 3	1 Bottle	Liquid	10 mL	Ready for use
96-well plate	1 Plate	NA	NA	Ready for use
Polypropylene cap	2 Sheets	NA	NA	Ready for use
Pre-slit cap mat	1 Sheet	NA	NA	Ready for use

Box 2 (storage at 18°C to 25°C)

Component	Quantity	Appearance	Content	Preparation prior to use
Reagent C	1 Vial	Dry powder	0.180 g	To be reconstituted with 3.35 mL of Ultrapure water

Box 3 (storage at -24°C to -18°C)

Component	Quantity	Appearance	Content	Preparation prior to use
Calibrators: 1,2,3,4,5,6	6 Vials	Liquid	250 μL	Ready for use
Blank	1 Vial	Liquid	1 mL	Ready for use
Quality Control samples:	9 Vials	Liquid	250 μL	Ready for use
Quality Control-0S CS Low/Medium/High				
Quality Control-4S CS Low/Medium/High				
Quality Control-6S CS Low/Medium/High				

UPLC-MS/MS Conditions

Waters® ACQUITY UPLC I-Class		
Column	Waters® ACQUITY UPLC-BEH C18 column, 1.7 µm, 2.1 x 100 mm	
Pre Column	Waters® ACQUITY UPLC BEH C18 VanGuard™ pre-column, 130Å, 1.7 µm, 2.1 mm x 5 mm	
Column Temperature	50 °C	
Sampler Temperature	10 °C	
Mobile Phase/Wash	A: 2% DMSO in 5 mM Ammonium Acetate solution B: 2% DMSO in Methanol Injection Wash: 30% Methanol in Ultrapure water Purge Solution: 10% Methanol in Ultrapure water Seal Wash Solution: 10% Methanol in Ultrapure water	
Flow Rate	0.5 mL/min	
Injection Volume	1 µL	
Gradient Program	Time (min)	B%
	0.00	5
	0.10	5
	4.00	25
	4.10	95
	5.00	95
	5.10	5
Run Time	6 min	
Waters® Xevo TQ-S micro		
Acquisition mode	Multiple Reaction Monitoring (MRM) - See Directions for Use for MRM Table	
Polarity	Electrospray in Negative Mode	
Capillary (kV)	0.5	
Source temp (°C)	150	
Desolvation temp (°C)	650	
Cone (V)	60.00	
Data Management	Waters®MassLynx SCN1045IVD, including Waters® TargetLynx (Part Number 667001791 Rev B)	

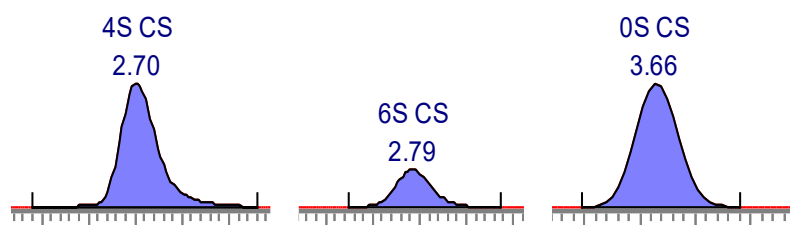


Figure 1. MS-Chromatogram. A typical MS-Chromatogram of the three target analytes 0S CS, 4S CS and 6S CS, respectively and their corresponding internal standards obtained using the Inlet/MS/Tune and Integration methods reported in the Directions for Use. Numbers represent retention time.

ANALYTICAL VALIDATION

Table 1. Summary of analytical validation studies, acceptance criteria and outcomes for each of the three analytes 0S CS, 4S CS and 6S CS, respectively. Note: the analytical validation results are presented here as an overall assessment against predefined acceptance criteria. Detailed quantitative data and statistical analyses have been omitted from this Application Note and will be reported separately in a planned peer-reviewed scientific publication.

Validation Characteristics	Acceptance Criteria	Outcome
Accuracy (Spike- Recovery)	Bias within $\pm 10\%$	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Assay Linearity	Deviation from linearity $\leq 10\%$	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
LLoQ	$\%TE \leq 20\%$	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Single-Site Precision (14-Day)	Total CV $\leq 15\%$ at low concentrations and $\leq 10\%$ at higher concentrations	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Operator to Operator Precision	Total CV $\leq 15\%$ at low concentrations and $\leq 10\%$ at higher concentrations	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Lot to Lot Precision	Total CV $\leq 15\%$ at low concentrations and $\leq 10\%$ at higher concentrations	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Site to Site Precision (2 sites)	Total CV $\leq 15\%$ at low concentrations and $\leq 10\%$ at higher concentrations	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Matrix effect	Response CV ≤ 15	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
Commutability	95% CI of matrix bias within $\pm 5\%$	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED
On-Board Stability	Drift $\leq 10\%$ ($\leq 15\%$ near LLoQ)	0S CS: PASSED, 4S CS: PASSED; 6S CS: PASSED. Analytes stable for 72h.
Carry-Over	For each disaccharide the carry-over is the highest concentration that does not carry over to the negative sample at or above 25% of the LLMI	No carry over observed.
Interference	Difference $\leq \pm 15\%$ between control and interferent spiked samples is considered non-significant	Study on-going. 11 substances tested. One substance, Glucose, shows interfering effects.

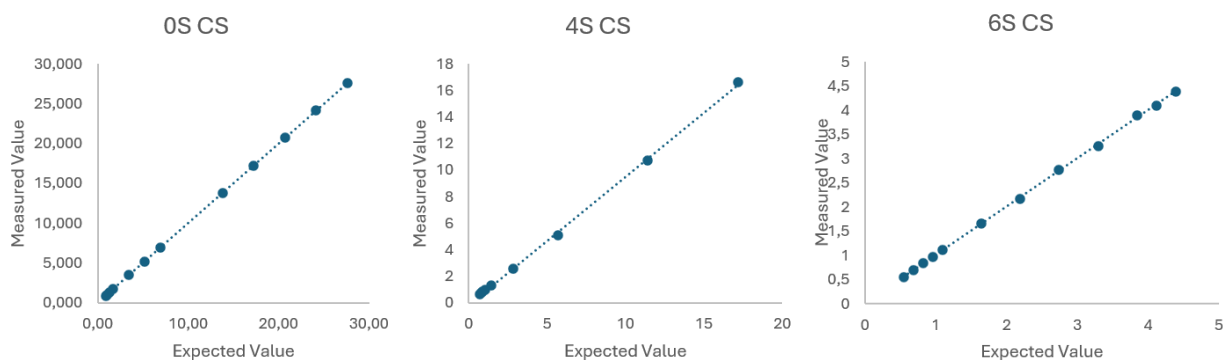


Figure 2. Linearity assessment for non-sulfated chondroitin sulfate (0S CS), chondroitin-4-sulfate (4S CS) and chondroitin-6-sulfate (6S CS), demonstrating assay linearity across the assay range.

DOCUMENTS AVAILABLE FOR DOWNLOAD

Directions For Use MIRAM GAGome Kit

Safety Data Sheet MIRAM GAGome Kit

MassLynx Project Files

www.elypta.com/e-resources

Register your account for access

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