ZYMO RESEARCH
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INNOVATIVE & QUALITY TOOLS
FOR EPIGENETICS RESEARCH AND DNA/RNA PURIFICATION

Mass Spectrometry Service for Global DNA Methylation and Hydroxymethylation Analysis

For

Customer

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1. Summary

An SRM-based mass spectrometry assay was used to quantify 5-hydroxymethyl-2'-deoxycytidine (5HmdC) and 5-methyl-2'-deoxycytidine (5mdC). The assay was designed to measure 5HmdC concentrations and 5mdC concentrations as a percentage of 2'-deoxyguanosine (dG) (e.g. – [5HmdC]/[dG] and [5mdC]/[dG]). The calibrated ranges for the analytes were 0-2.5% for 5HmdC and 0-25% for 5mdC using a fixed 40 pmol amount of dG as an internal standard.

Calibration point #6 was excluded from the 5HmdC calibration curve and calibration point #5 was excluded from the 5mdC calibration curve. These calibration points were excluded because they either had measured values which deviated more than 10% from the expected true value, or their removal/exclusion from the curve substantially improved the r2 value. The percent difference for all of the included calibration points for all curves did not exceed 6% from the specified true amounts of measured analyte. The calibration points were run as single replicates due to previously demonstrated high reproducibility of the assay.

The samples had a measured range of 5HmdC as low as 1.59% and as high as 2.67%. The samples had a measured range of 5mdC between 7.84% and 9.29%. Replicates for the unknown samples were run in triplicate followed by a blank to eliminate carryover into the next unknown run.

2. Data Report

2.1. Services Performed

The following checklist confirms the steps of the Zymo Research Epigenetic Services that were performed on your samples.

SERVICE	
Sample Received	✓
Sample Quality Evaluated	✓
Sample Prepared for MS	✓
MS Performed	✓
Data/Results	✓

2.2. Sample IDs

1. ZR_1
2. ZR_2
3. ZR_3
4. ZR_4

2.3. Terminology

Area: Area under the peak for either 5mdC or 5HmdC
ISTD Area: Area under the peak for dG
Area Ratio: Ratio of Area to ISTD Area (=Area/ISTD Area)
Calculated Amt (%): Percent of either 5mdC or 5HmdC is calculated using the area ratio and standard curves in the range of 0-2.5% 5HmdC/dG or 0-25% 5mdC/dG
RT (min): Retention time- amount of time it took for the peak corresponding to the modification to come off the column

2.4. Data

Table 1: Calculated concentrations of endogenous 5HmdC (note: NF = not found)

Sample	Area	ISTD Area	Area Ratio	Retention Time	Calculated %5HmdC
ZR_1 rep 1	8799	583911	0.015	2.37	1.90
ZR_1 rep 2	9447	592640	0.016	2.39	2.01
ZR_1 rep 3	9714	605404	0.016	2.39	2.03
ZR_2 rep 1	9491	730577	0.013	2.39	1.64
ZR_2 rep 2	9495	753119	0.013	2.37	1.59
ZR_2 rep 3	9787	745833	0.013	2.37	1.66
ZR_3 rep 1	16670	797323	0.021	2.37	2.64
ZR_3 rep 2	17460	846700	0.021	2.39	2.60
ZR_3 rep 3	17475	825153	0.021	2.39	2.67
ZR_4 rep 1	12157	624211	0.019	2.37	2.46
ZR_4 rep 2	12382	635877	0.019	2.39	2.46
ZR_4 rep 3	12236	622265	0.020	2.37	2.48

The order of samples listed in the table above reflects the run order in which the data was acquired.

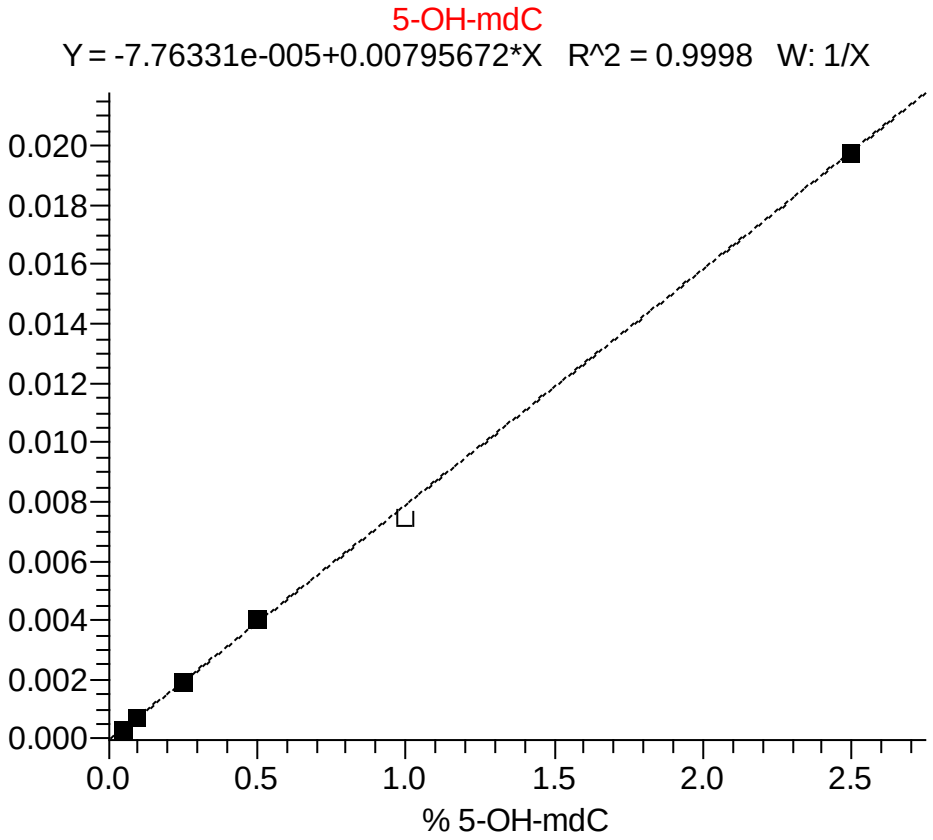
Table 2: Calculated concentrations of endogenous 5mdC (note: NF = not found)

Sample	Area	ISTD Area	Area Ratio	Retention Time	Calculated %5mdC
ZR_1 rep 1	96712	583911	0.166	3.61	8.94
ZR_1 rep 2	102055	592640	0.172	3.63	9.29
ZR_1 rep 3	103705	605404	0.171	3.61	9.24
ZR_2 rep 1	122225	730577	0.167	3.63	9.03
ZR_2 rep 2	123421	753119	0.164	3.61	8.84
ZR_2 rep 3	122232	745833	0.164	3.63	8.84
ZR_3 rep 1	124862	797323	0.157	3.61	8.45
ZR_3 rep 2	122984	846700	0.145	3.61	7.84
ZR_3 rep 3	127143	825153	0.154	3.63	8.31
ZR_4 rep 1	96769	624211	0.155	3.61	8.37
ZR_4 rep 2	95664	635877	0.150	3.61	8.12
ZR_4 rep 3	96890	622265	0.156	3.61	8.40

The order of samples listed in the table above reflects the run order in which the data was acquired.

2.5. Standard Curves for 5HmdC and 5mdC

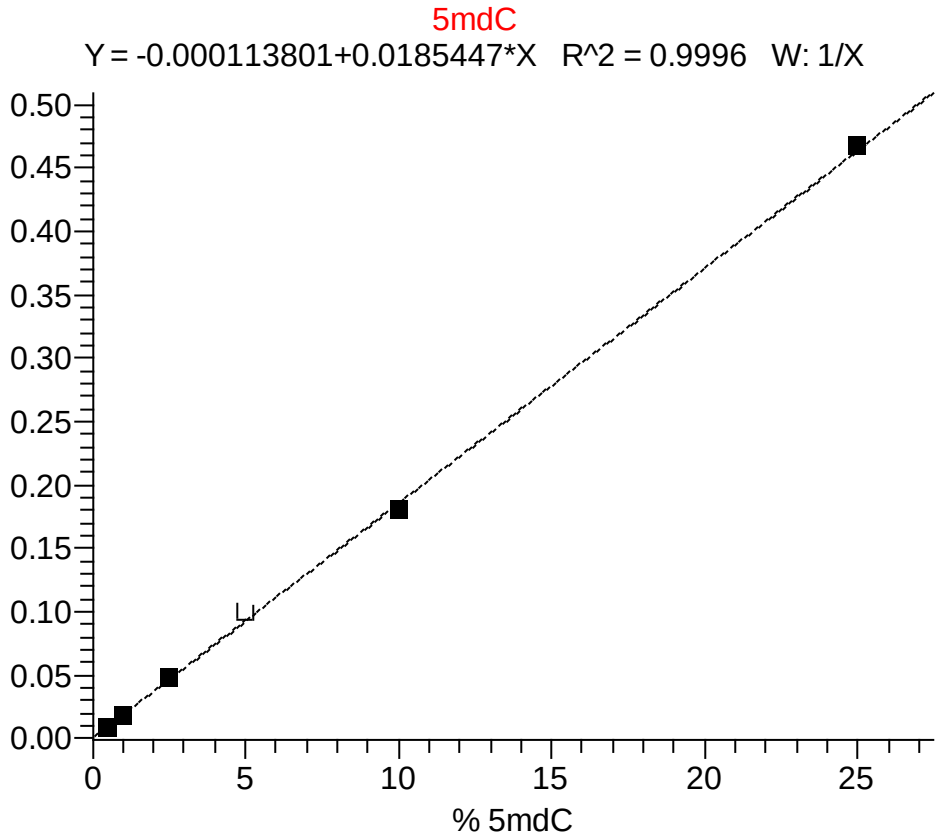
2.5.1. 5HmdC calibration curve over the range of 0-2.5% [5HmdC]/[dG]:



Sample Name	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	RT
cal1_01	NF	840527	NF	0.000	NF	NF
cal2_01	266	834856	0.000	0.050	0.050	2.39
cal3_01	612	870948	0.001	0.100	0.098	2.41
cal4_01	1584	830417	0.002	0.250	0.250	2.41
cal5_01	3267	812964	0.004	0.500	0.515	2.39
cal6_01	6394	859433	0.007	1.000	0.945	2.39
cal7_01	16283	825843	0.020	2.500	2.488	2.39
QC-check_01	3301	836477	0.004	NA	0.506	2.37
QC-check_02	3449	816022	0.004	NA	0.541	2.39

Note: Calibration point #6 was excluded from the curve.

2.5.2. 5mdC calibration curve over the range of 0-25% [5mdC]/[dG]:



Sample Name	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	RT
cal1_01	NF	840527	NF	0.000	NF	NF
cal2_01	7462	834856	0.009	0.500	0.488	3.61
cal3_01	16309	870948	0.019	1.000	1.016	3.61
cal4_01	39476	830417	0.048	2.500	2.570	3.63
cal5_01	81461	812964	0.100	5.000	5.409	3.61
cal6_01	154776	859433	0.180	10.000	9.717	3.63
cal7_01	385984	825843	0.467	25.000	25.209	3.63
QC-check_01	82805	836477	0.099	NA	5.344	3.61
QC-check_02	79979	816022	0.098	NA	5.291	3.63

Note: Calibration point #5 was excluded from the curve.

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