



A-YES®

YEAST CELL ASSAY FOR THE DETECTION OF ESTROGENIC ACTIVITY

CERTIFICATE OF ANALYSIS

YEAST LOT NUMBER 64AURA

DATE OF EXPERIMENT 2020-05-15

DATE OF STATISTICAL ANALYSIS 2020-05-15

(FILE) NAME OF ANALYSIS Report

NUMBER OF ANALYSED SAMPLES 7

NUMBER OF REPLICATES/WELLS 2 - 4

ANALYSED SAMPLES

Sample	Internal Sample Code	Notes
S1		
S2		
S3		
S4		
S5		
S6		
S7		

OPERATOR _____

RESPONSIBLE PERSON _____

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Pipetting Scheme and Raw Data

Type		1	2	3	4	5	6	7	8	9	10	11	12
	A	NC	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	NC
	B	CS	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
	C	CS	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
	D	CS	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
	E	CS	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
	F	CS	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
	G	CS	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
	H	CS	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS

Spike [ng/L]		1	2	3	4	5	6	7	8	9	10	11	12	
	A	0	0	0	0	0	0	0	0	0	0	0	0	0
	B	1	0	0	0	0	0	0	0	0	0	0	0	1
	C	2	0	0	0	0	0	0	0	0	0	0	0	2
	D	4	0	0	0	0	0	0	0	0	0	0	0	4
	E	8	0	0	0	0	0	0	0	0	0	0	0	8
	F	20	0	0	0	0	0	0	0	0	0	0	0	20
	G	40	0	0	0	0	0	0	0	0	0	0	0	40
	H	80	0	0	0	0	0	0	0	0	0	0	0	80

Sample Dilution		1	2	3	4	5	6	7	8	9	10	11	12
	A	0	10	10	10	10	10	25	25	25	25	200	0
	B	0	20	20	20	20	20	50	50	50	50	400	0
	C	0	50	50	50	50	50	75	75	75	75	800	0
	D	0	100	100	100	100	100	100	100	100	100	1000	0
	E	0	10	10	10	5	5	25	25	25	25	200	0
	F	0	20	20	20	10	10	50	50	50	50	400	0
	G	0	50	50	50	20	20	75	75	75	75	800	0
	H	0	100	100	100	50	50	100	100	100	100	1000	0

OD Phytase		1	2	3	4	5	6	7	8	9	10	11	12
	A	0.322	1.848	1.873	1.827	2.792	2.722	0.373	0.421	0.395	0.361	0.362	0.313
	B	0.334	1.255	1.215	1.241	1.686	1.643	0.376	0.369	0.396	0.378	0.354	0.323
	C	0.329	0.685	0.664	0.653	0.711	0.701	0.366	0.400	0.375	0.376	0.383	0.325
	D	0.398	0.448	0.452	0.433	0.456	0.430	0.363	0.370	0.369	0.392	0.350	0.376
	E	0.634	2.148	2.133	2.097	1.232	1.207	0.366	0.364	0.362	0.364	0.357	0.643
	F	1.928	1.625	1.596	1.589	0.815	0.790	0.363	0.359	0.363	0.349	0.364	1.950
	G	3.257	0.876	0.869	0.877	0.546	0.511	0.372	0.363	0.356	0.368	0.353	3.354
	H	3.661	0.566	0.563	0.518	0.424	0.345	0.366	0.358	0.366	0.376	0.367	3.635

OD Growth		1	2	3	4	5	6	7	8	9	10	11	12
	A	0.463	1.020	1.052	1.053	0.872	0.887	0.215	0.226	0.191	0.188	0.321	0.513
	B	0.523	0.921	0.926	0.916	0.739	0.748	0.271	0.271	0.233	0.231	0.283	0.563
	C	0.476	0.697	0.736	0.758	0.613	0.624	0.375	0.246	0.270	0.228	0.286	0.525
	D	0.546	0.642	0.717	0.698	0.614	0.641	0.218	0.245	0.258	0.194	0.285	0.535
	E	0.562	1.000	0.997	1.003	0.991	1.001	0.209	0.263	0.211	0.266	0.251	0.642
	F	0.531	0.807	0.793	0.847	0.873	0.853	0.220	0.220	0.223	0.279	0.304	0.527
	G	0.521	0.661	0.636	0.642	0.815	0.744	0.189	0.219	0.215	0.245	0.292	0.583
	H	0.453	0.585	0.601	0.647	0.770	0.675	0.171	0.252	0.198	0.240	0.256	0.534

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Statistical Assessment

Growth Density

Check of Yeast Growth Effects between Samples and Calibration Standards

No check of yeast growth effects was carried out.

Calibration Levels

Check of Outliers within Calibration Levels

No single values could be identified as outliers.

Check of Variability within Calibration Levels

OK!

Samples

Check of Outliers within Samples

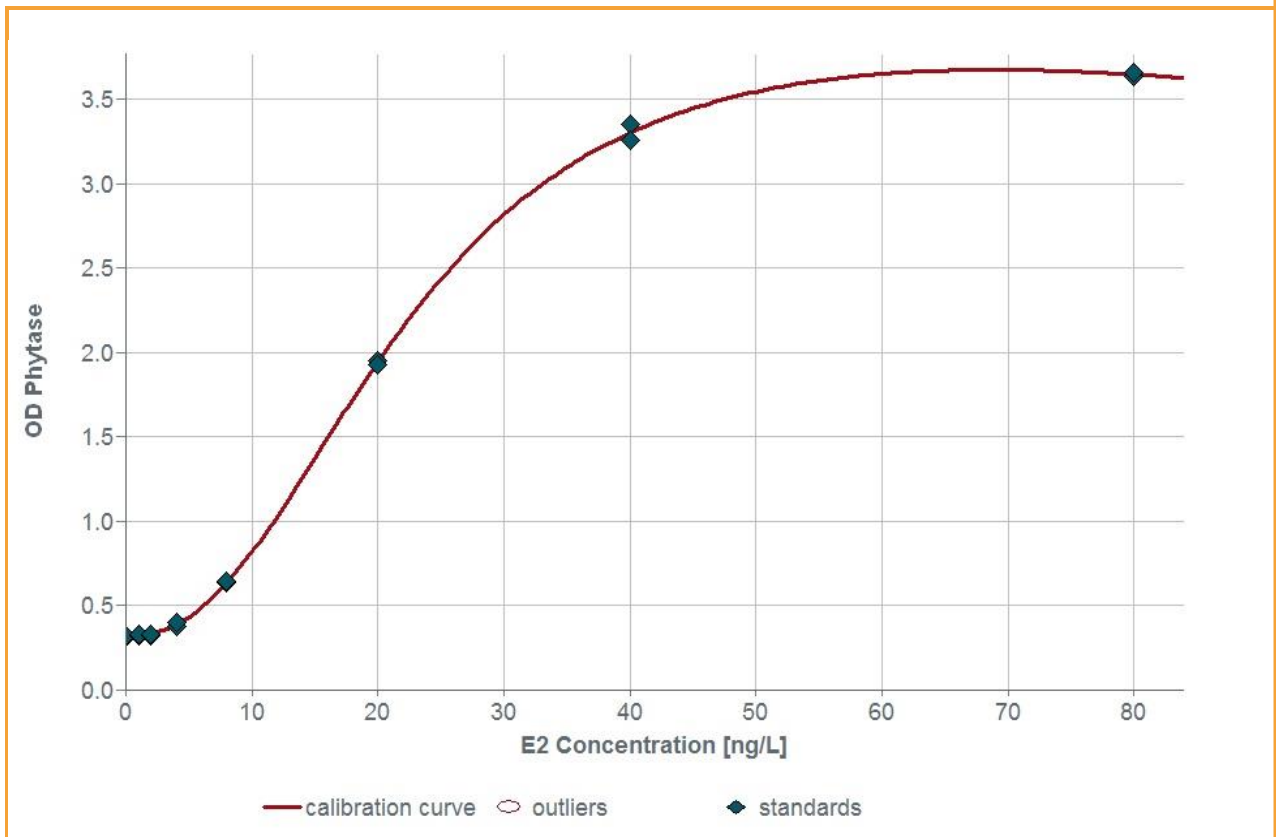
No single values could be identified as outliers.

Check of Variability within Samples

OK!

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Calibration Curve



Characterization of Calibration Curve

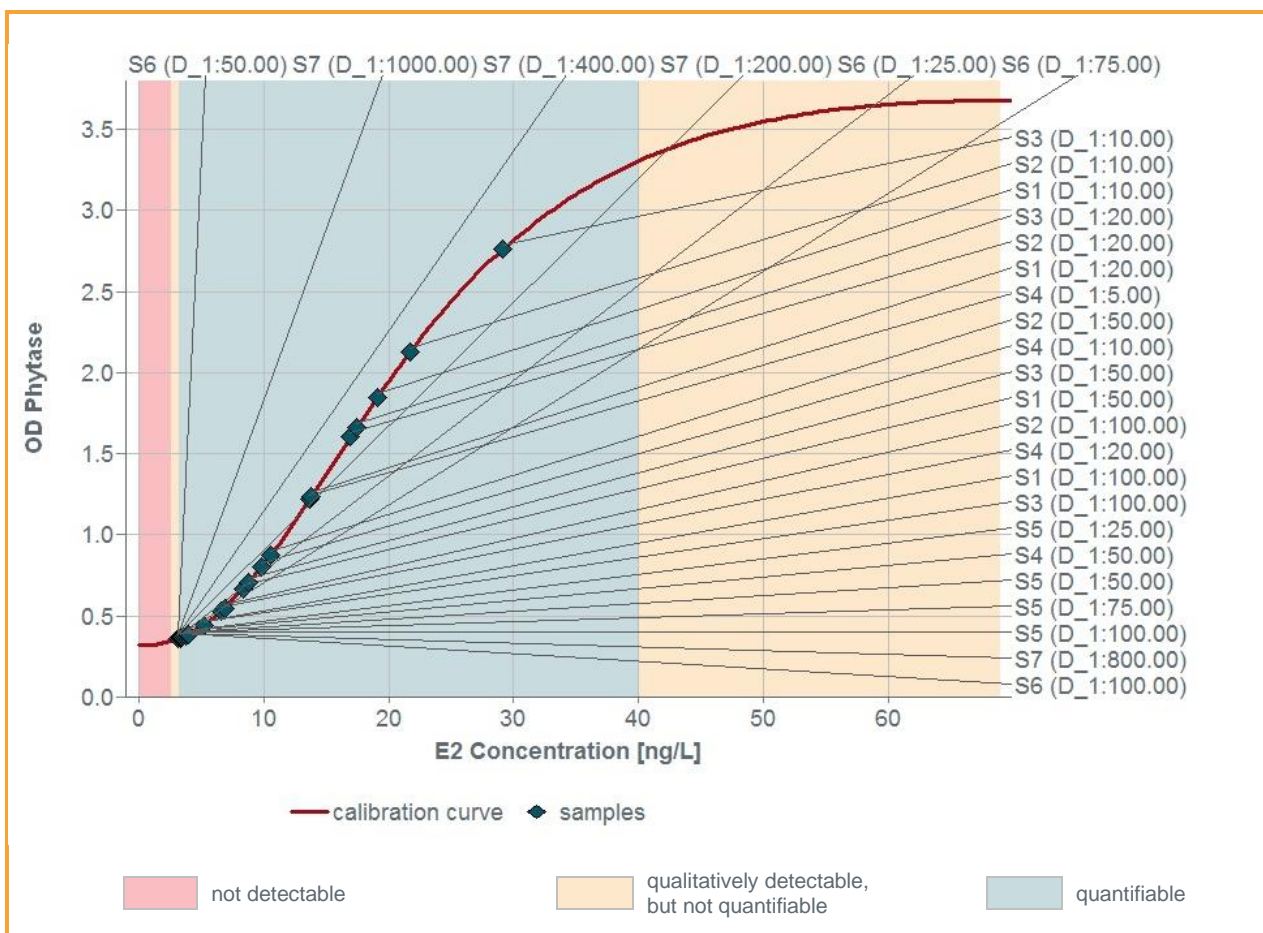
Expected Optical Density for the Blank Value	A	0.319	OK
Slope Parameter of the Curve	B	2.588	OK
Center of the Test [ng/L]	C	20.493	OK
Maximum Optical Density	D	3.676	OK
Variability of Residuals		2.13 %	OK
Sensitivity Range Characteristic		11.5	OK
Coverage of Calibration Range		100.0 %	OK
Check of Homogeneity of Relative Variances			OK

Quality Control Parameters of Calibration Curve

Limit of Detection		2.57 ng/L
Limits of Quantification	Lower Limit	3.19 ng/L
	Upper Limit	40.02 ng/L

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Reporting of Results of Sample Analysis



Summary Results of Sample Analysis

Sample	Sample Dilution	Spiked E2 Concentration [ng/L]	Diluted and Spiked Sample		Undiluted and Unspiked Sample	
			Qualitatively Detected	Quantifiable	E2 Equivalents (EEQ) [ng/L]	Measurement Uncertainty
S1	1:100.00	0.0	yes	yes	521.9	7.2 %
S1	1:50.00	0.0	yes	yes	418.9	4.4 %
S1	1:20.00	0.0	yes	yes	276.5	3.7 %
S1	1:10.00	0.0	yes	yes	191.3	4.1 %
S2	1:100.00	0.0	yes	yes	688.9	5.2 %
S2	1:50.00	0.0	yes	yes	527.3	3.8 %
S2	1:20.00	0.0	yes	yes	339.2	3.9 %
S2	1:10.00	0.0	yes	yes	217.4	4.5 %
S3	1:100.00	0.0	yes	yes	519.4	7.2 %
S3	1:50.00	0.0	yes	yes	440.8	4.2 %
S3	1:20.00	0.0	yes	yes	349.8	4.0 %
S3	1:10.00	0.0	yes	yes	291.4	6.7 %

CERTIFICATE OF ANALYSIS

Reporting of Results of Sample Analysis

Sample	Sample Dilution	Spiked E2 Concentration [ng/L]	Diluted and Spiked Sample		Undiluted and Unspiked Sample	
			Qualitatively Detected	Quantifiable	E2 Equivalents (EEQ) [ng/L]	Measurement Uncertainty
S4	1:50.00	0.0	yes	yes	195.3	11.2 %
S4	1:20.00	0.0	yes	yes	131.9	5.4 %
S4	1:10.00	0.0	yes	yes	98.4	3.9 %
S4	1:5.00	0.0	yes	yes	68.4	3.7 %
S5	1:100.00	0.0	yes	yes	360.1	13.0 %
S5	1:75.00	0.0	yes	yes	282.3	11.9 %
S5	1:50.00	0.0	yes	yes	188.9	11.9 %
S5	1:25.00	0.0	yes	yes	99.6	10.8 %
S6	1:100.00	0.0	yes	yes	338.9	14.6 %
S6	1:75.00	0.0	yes	yes	250.0	15.2 %
S6	1:50.00	0.0	yes	no	156.3	17.5 %
S6	1:25.00	0.0	yes	yes	82.7	15.4 %
S7	1:1000.00	0.0	yes	no	3125.6	17.5 %
S7	1:800.00	0.0	yes	yes	2749.0	14.2 %
S7	1:400.00	0.0	yes	no	1257.1	17.3 %
S7	1:200.00	0.0	yes	no	632.0	17.1 %

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Detailed Results of Sample Analysis

Sample	Sample Dilution	Spiked E2 Concentration [ng/L]	OD Phytase		E2 Equivalentents (EEQ) in ng/L for		Measurement Uncertainty for Undiluted and Unspiked Sample
			Mean Value *)	Relative S. D. *)	Diluted and Spiked Sample	Undiluted and Unspiked Sample	
S1	1:100.00	0.0	0.444	2.3 %	5.2	521.9	7.2 %
S1	1:50.00	0.0	0.667	2.4 %	8.4	418.9	4.4 %
S1	1:20.00	0.0	1.237	1.6 %	13.8	276.5	3.7 %
S1	1:10.00	0.0	1.849	1.2 %	19.1	191.3	4.1 %
S2	1:100.00	0.0	0.549	4.9 %	6.9	688.9	5.2 %
S2	1:50.00	0.0	0.874	0.5 %	10.5	527.3	3.8 %
S2	1:20.00	0.0	1.603	1.2 %	17.0	339.2	3.9 %
S2	1:10.00	0.0	2.126	1.2 %	21.7	217.4	4.5 %
S3	1:100.00	0.0	0.443	4.2 %	5.2	519.4	7.2 %
S3	1:50.00	0.0	0.706	1.0 %	8.8	440.8	4.2 %
S3	1:20.00	0.0	1.664	1.8 %	17.5	349.8	4.0 %
S3	1:10.00	0.0	2.757	1.8 %	29.1	291.4	6.7 %
S4	1:50.00	0.0	0.384	14.5 %	3.9	195.3	11.2 %
S4	1:20.00	0.0	0.528	4.7 %	6.6	131.9	5.4 %
S4	1:10.00	0.0	0.802	2.2 %	9.8	98.4	3.9 %
S4	1:5.00	0.0	1.220	1.4 %	13.7	68.4	3.7 %
S5	1:100.00	0.0	0.373	3.4 %	3.6	360.1	13.0 %
S5	1:75.00	0.0	0.379	3.8 %	3.8	282.3	11.9 %
S5	1:50.00	0.0	0.380	3.0 %	3.8	188.9	11.9 %
S5	1:25.00	0.0	0.388	6.8 %	4.0	99.6	10.8 %
S6	1:100.00	0.0	0.366	2.0 %	3.4	338.9	14.6 %
S6	1:75.00	0.0	0.365	1.9 %	3.3	250.0	15.2 %

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Detailed Results of Sample Analysis

Sample	Sample Dilution	Spiked E2 Concentration [ng/L]	OD Phytase		E2 Equivalents (EEQ) in ng/L for		Measurement Uncertainty for Undiluted and Unspiked Sample
			Mean Value ^{*)}	Relative S. D. ^{*)}	Diluted and Spiked Sample	Undiluted and Unspiked Sample	
S6	1:50.00	0.0	0.358	1.8 %	3.1	156.3	17.5 %
S6	1:25.00	0.0	0.364	0.4 %	3.3	82.7	15.4 %
S7	1:1000.00	0.0	0.358	3.4 %	3.1	3125.6	17.5 %
S7	1:800.00	0.0	0.368	5.8 %	3.4	2749.0	14.2 %
S7	1:400.00	0.0	0.359	2.0 %	3.1	1257.1	17.3 %
S7	1:200.00	0.0	0.359	1.0 %	3.2	632.0	17.1 %

^{*)} The mean value and the relative standard deviation are based on the growth-corrected raw data if applicable.