



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 \_\_\_\_\_

**CERTIFICATE OF ANALYSIS**

## Pipetting Scheme and Raw Data

Type		1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S1	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	NC
B	CS	S1	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
C	CS	S1	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
D	CS	S1	S1	S1	S1	S3	S3	S5	S5	S5	S5	S7	CS
E	CS	S2	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
F	CS	S2	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
G	CS	S2	S2	S2	S2	S4	S4	S6	S6	S6	S6	S7	CS
H	CS	S2	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	10	25	25	25	25	200	0
B	0	20	20	20	20	20	20	50	50	50	50	400	0
C	0	50	50	50	50	50	50	75	75	75	75	800	0
D	0	100	100	100	100	100	100	100	100	100	100	1000	0
E	0	10	10	10	10	5	5	25	25	25	25	200	0
F	0	20	20	20	20	10	10	50	50	50	50	400	0
G	0	50	50	50	50	20	20	75	75	75	75	800	0
H	0	100	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	

**CERTIFICATE OF ANALYSIS**

## 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*

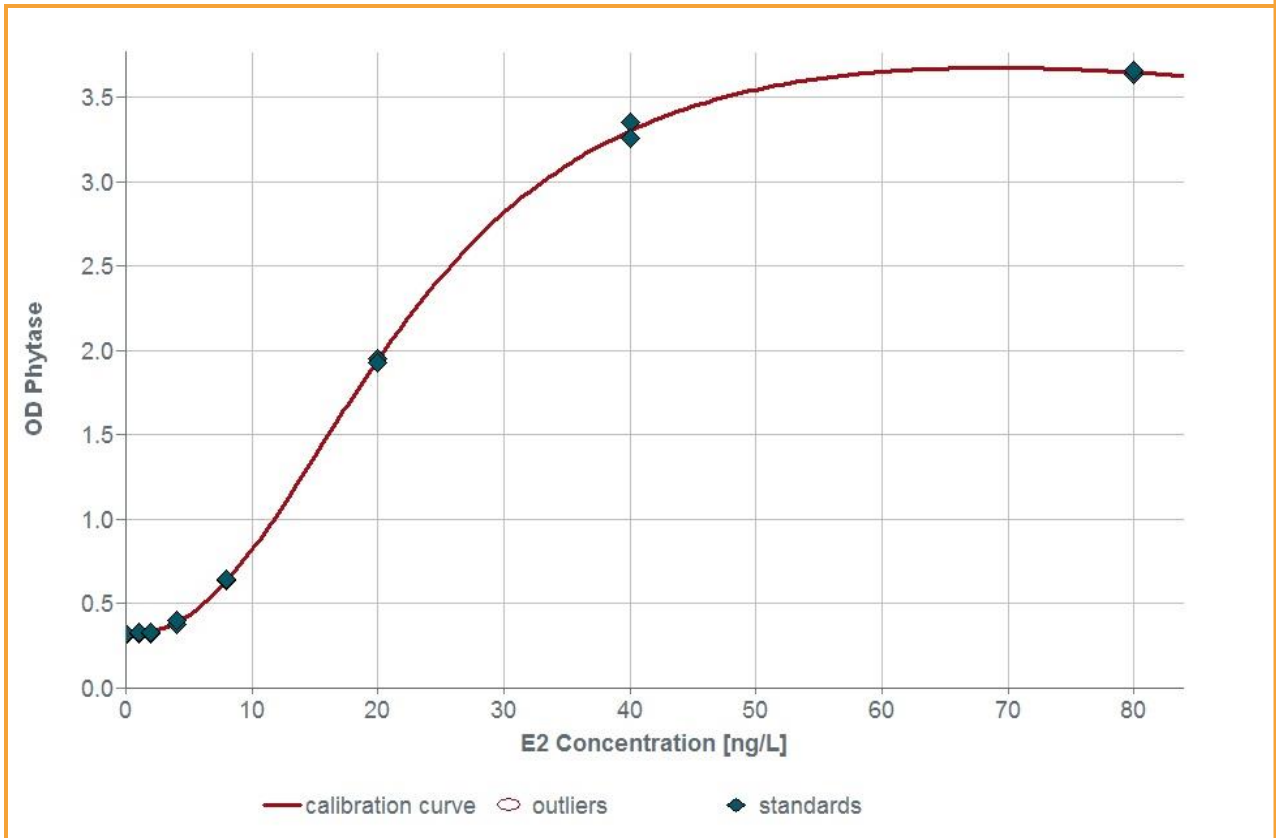
No single values could be identified as outliers.

*Check of Variability within Samples*

OK!

# CERTIFICATE OF ANALYSIS

## Calibration Curve



### Characterization of Calibration Curve

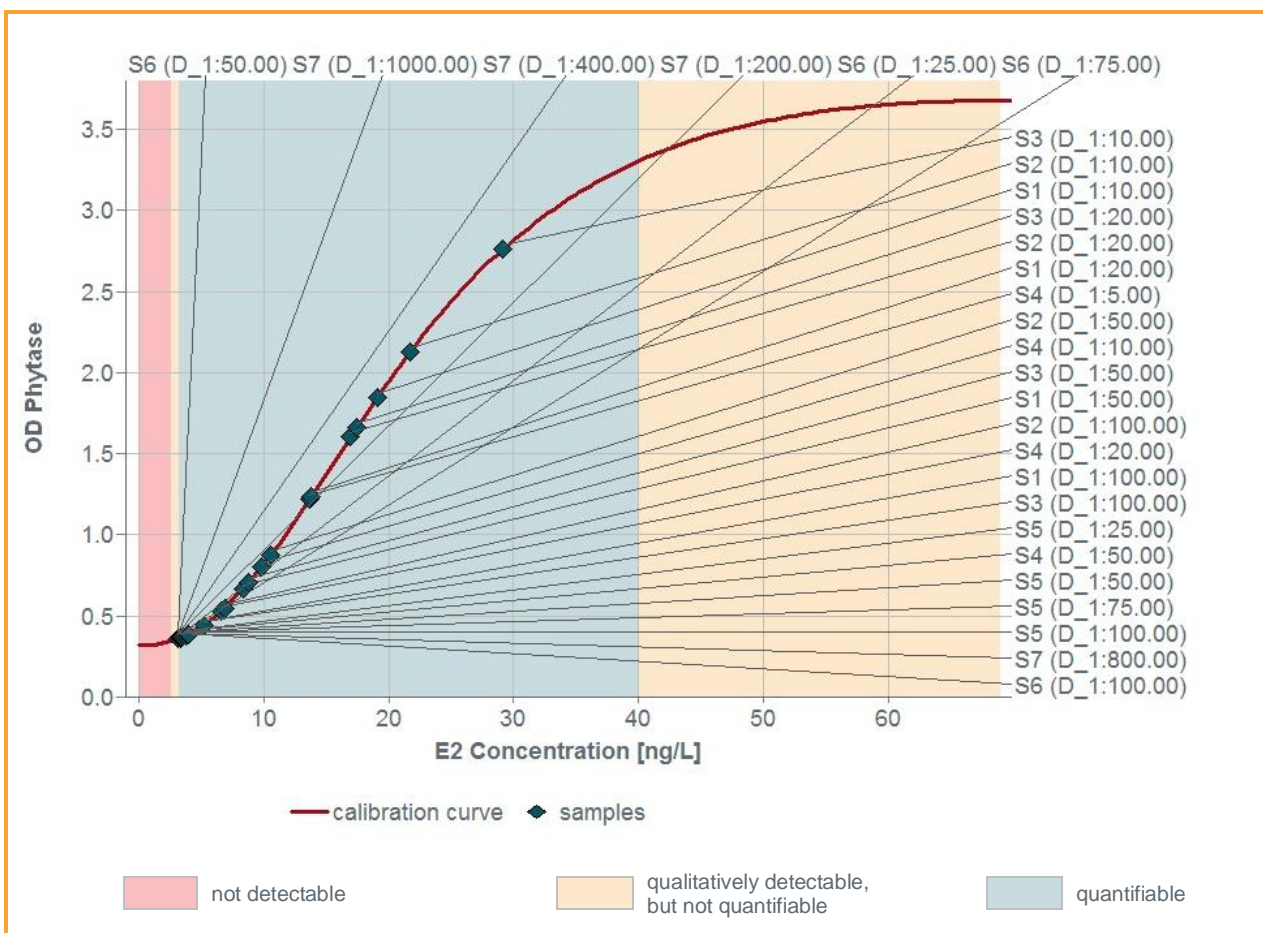
Expected Optical Density for the Blank Value	A	0.319	OK
Slope Parameter of the Curve	B	2.588	OK
Center of the Test [ng/L]	C	20.493	OK
Maximum Optical Density	D	3.676	OK
Variability of Residuals		2.13 %	OK
Sensitivity Range Characteristic		11.5	OK
Coverage of Calibration Range		100.0 %	OK
Check of Homogeneity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

# CERTIFICATE OF ANALYSIS

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 %

**CERTIFICATE OF ANALYSIS**

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	
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 %

**CERTIFICATE OF ANALYSIS**

## 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 <sup>*)</sup>	Relative S. D. <sup>*)</sup>	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 %

<sup>\*)</sup> The mean value and the relative standard deviation are based on the growth-corrected raw data if applicable.