

日本薬局方エンドトキシン標準品 現行製品及び新製品（エンドトキシン 10000）の測定結果

日局エンドトキシン標準品の新製品への切替に際して、一般社団法人 医薬品医療機器レギュラトリーサイエンス財団（PMRJ）より依頼を受け、弊社製品を用いて、日局エンドトキシン標準品現行製品及び新製品（エンドトキシン 10000）を測定しました。下記にその結果をお示しします。

記

【方法】

日局エンドトキシン標準品の現行製品及び新製品をエンドトキシン試験用水で溶解し、標準原液を調製した。標準原液それぞれを希釈してエンドトキシン標準溶液を調製し、各ライセート試薬（及び遺伝子組換えタンパク質を用いたエンドトキシン測定試薬）で測定した。得られた結果から、用量反応直線を作成した。

標準品

日局エンドトキシン標準品現行製品（Lot ENH29A） 16000 EU/瓶

日局エンドトキシン標準品新製品（Lot N-ENH01A（サンプル品を PMRJ より受領）） 10000 EU/瓶

試薬

1. 比色法：Kinetic-QCL™
測定範囲；0.005~50EU/mL
2. 比濁法：Pyrogent™-5000
測定範囲；0.01~100EU/mL
3. 蛍光法（欧州薬局方 Chapter2.6.32 収載）：PyroGene™ r Fc
測定範囲；0.005~5.0EU/mL

操作方法

4. 用手法
①各ライセート試薬 3 ロットによる検量線の信頼性確認試験
5. エンドトキシン自動測定システム PyroTec Pro™
②検量線の信頼性確認試験

【結果】

6. 比色法：Kinetic-QCL™

検量線濃度；0.005-0.05-0.5-5.0-50 EU/mL (5濃度)

結果：表1~3、図1~8

① 日本薬局方エンドトキシン標準品 従来ロット

日本薬局方エンドトキシン標準品：ENH29A
使用ライセート試薬：Kinetic-QCL (比色法)

表1

LOT	Concentration (EU/mL)					Correlation Coefficient	Slope	Y Intercept
	0.005	0.05	0.5	5.0	50			
YL038GRLX8	3973	2418	1294	748	448	-0.999	-0.240	3.053
	3905	2452	1317	751	449			
	3919	2439	1322	749	448			
YL101L6PQB	3230	2057	1128	642	383	-0.999	-0.236	2.984
	3226	2086	1151	648	387			
	3268	2063	1149	632	387			
YL121HVBA3	3379	2254	1216	691	428	-0.998	-0.231	3.019
	3426	2270	1222	724	430			
	3421	2189	1198	687	424			



図1：YL038GRLX8

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.999 Slope = -0.240 Y Intercept = 3.053

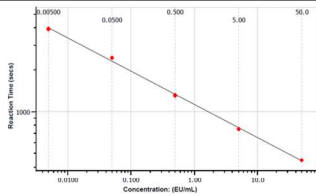


図2：YL101L6PQB

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.999 Slope = -0.236 Y Intercept = 2.984

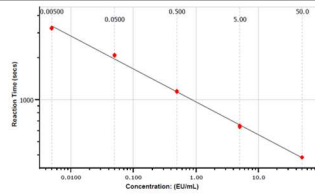
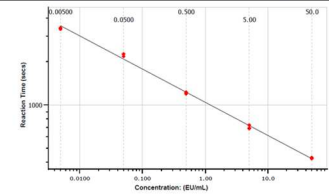


図3：YL121HVBA3

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.998 Slope = -0.231 Y Intercept = 3.019



② 日本薬局方エンドトキシン標準品 新ロット

日本薬局方エンドトキシン標準品：N-ENH01A
使用ライセート試薬：Kinetic-QCL (比色法)

表2

LOT	Concentration (EU/mL)					Correlation Coefficient	Slope	Y Intercept
	0.005	0.05	0.5	5	50			
YL038GRLX8	3300	1700	935	518	299	-1.000	-0.259	2.907
	3265	1721	958	529	301			
	3307	1707	950	536	301			
YL101L6PQB	2713	1450	814	460	251	-1.000	-0.256	2.84
	2732	1459	816	455	262			
	2911	1559	872	484	287			
YL121HVBA3	2932	1573	875	492	291	-0.999	-0.253	2.873
	2934	1558	861	479	283			

図4：YL038GRLX8

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -1.000 Slope = -0.259 Y Intercept = 2.907

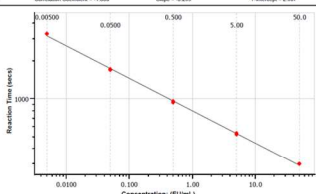


図5：YL101L6PQB

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -1.000 Slope = -0.256 Y Intercept = 2.84

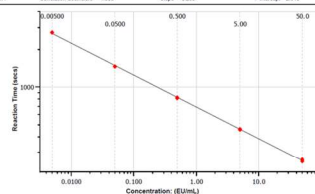
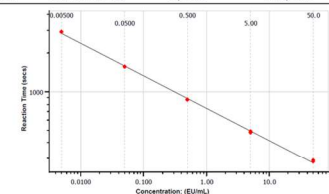


図6：YL121HVBA3

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 36.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.999 Slope = -0.253 Y Intercept = 2.873



③ エンドトキシン自動測定システム PyroTec Pro

日本薬局方エンドトキシン標準品：ENH29A, N-ENH01A
使用ライセート試薬：Kinetic-QCL (Lot: YL121HVBA3)

表3

LOT	Concentration (EU/mL)					Correlation Coefficient	Slope	Y Intercept
	0.005	0.05	0.5	5	50			
ENH29A	3482	2205	1164	667	392	-0.999	-0.241	3.004
	3447	2214	1179	669	393			
	3516	2254	1202	675	395			
N-ENH01A	3462	2156	1152	663	390	-0.999	-0.241	3.000
	3406	2167	1163	666	392			
	3524	2196	1198	670	395			

図7：ENH29A

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 37.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.999 Slope = -0.241 Y Intercept = 3.004

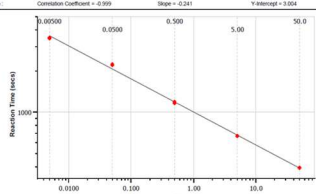
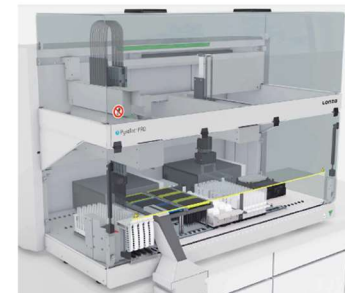
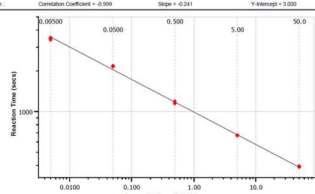


図8：N-ENH01A

Performed by: SUPERIOR
QCCL - Initial Qualification Test
Temperature: 37.0°C - 37.0°C
Linear Regression: Correlation Coefficient = -0.999 Slope = -0.241 Y Intercept = 3.000



7. 比濁法 : Pyrogen™-5000

検量線濃度 ; 0.01-0.1-1.0-10-100 EU/mL (5 濃度)

結果 : 表 4~6、図 9~16

① 日本薬局方エンドトキシン標準品 従来ロット

日本薬局方エンドトキシン標準品 : ENH29A
使用ライセート試薬 : Pyrogen-5000 (比濁法)

表 4

LOT	0.01	0.1	1.0	10	100
YL039E72VQ	2640	1280	644	362	205
	2695	1263	643	368	205
	2610	1257	646	367	204
YL097WHBYY	2565	1262	652	375	228
	2680	1266	657	375	228
	2595	1250	642	375	226
YL109VHKAQ	2730	1310	685	409	251
	2765	1302	680	405	253
	2840	1320	684	401	252

Correlation Coefficient	Slope	Y Intercept
-1.000 to -0.980	-0.400 to -0.100	2,500 to 3,500
-0.998	-0.276	2,842
-0.997	-0.265	2,852
-0.995	-0.259	2,881



図9 : YL039E72VQ

Performed by: SUPERVISOR LAL Lot No.: YL039E72VQ Exp: 4/20/2024 Time: 10:04:55AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: ENH29A Endotoxin Lot No.: ENH29A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.998 Slope = -0.276 Y Intercept = 2,842

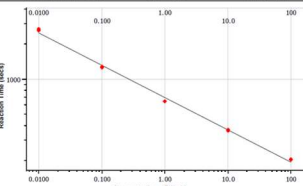


図10 : YL097WHBYY

Performed by: SUPERVISOR LAL Lot No.: YL097WHBYY Exp: 3/28/2024 Time: 11:03:55AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: ENH29A Endotoxin Lot No.: ENH29A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.997 Slope = -0.265 Y Intercept = 2,852

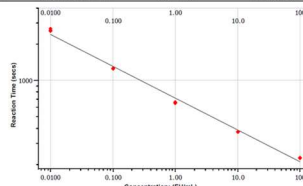
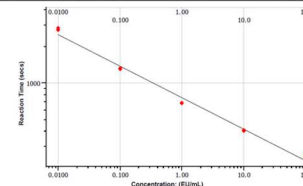


図11 : YL109VHKAQ

Performed by: SUPERVISOR LAL Lot No.: YL109VHKAQ Exp: 11/18/2024 Time: 10:04:55AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: ENH29A Endotoxin Lot No.: ENH29A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.995 Slope = -0.259 Y Intercept = 2,881



② 日本薬局方エンドトキシン標準品 新ロット

日本薬局方エンドトキシン標準品 : N-ENH01A
使用ライセート試薬 : Pyrogen-5000 (比濁法)

表 5

LOT	0.01	0.1	1	10	100
YL039E72VQ	2560	1220	628	357	207
	2485	1218	634	364	207
	2510	1213	629	363	204
YL097WHBYY	2535	1219	633	368	226
	2530	1223	636	366	226
	2525	1205	635	363	225
YL109VHKAQ	2610	1245	658	392	244
	2665	1242	659	393	243
	2690	1254	652	388	241

Correlation Coefficient	Slope	Y Intercept
-1.000 to -0.980	-0.400 to -0.100	2,500 to 3,500
-0.998	-0.270	2,832
-0.996	-0.262	2,841
-0.995	-0.258	2,863

図12 : YL039E72VQ

Performed by: SUPERVISOR LAL Lot No.: YL039E72VQ Exp: 4/20/2024 Time: 11:43:31AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: N-ENH01A Endotoxin Lot No.: N-ENH01A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.998 Slope = -0.270 Y Intercept = 2,832

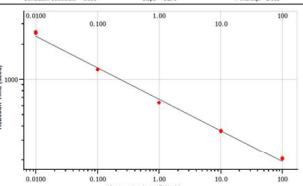


図13 : YL097WHBYY

Performed by: SUPERVISOR LAL Lot No.: YL097WHBYY Exp: 3/28/2024 Time: 11:03:55AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: N-ENH01A Endotoxin Lot No.: N-ENH01A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.996 Slope = -0.262 Y Intercept = 2,841

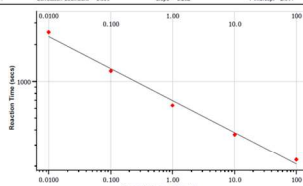
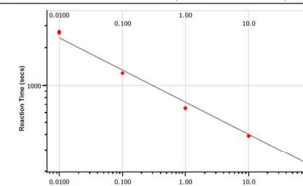


図14 : YL109VHKAQ

Performed by: SUPERVISOR LAL Lot No.: YL109VHKAQ Exp: 11/18/2024 Time: 11:43:31AM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: N-ENH01A Endotoxin Lot No.: N-ENH01A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.995 Slope = -0.258 Y Intercept = 2,863



③ エンドトキシン自動測定システム PyroTec Pro

日本薬局方エンドトキシン標準品 : ENH29A, N-ENH01A
使用ライセート試薬 : Pyrogen-5000 (比濁法)

表 6

LOT	0.01	0.1	1	10	100
ENH29A	2640	1311	675	387	215
	2637	1272	682	388	217
	2550	1277	669	385	215
N-ENH01A	2500	1260	656	391	221
	2537	1235	657	379	225
	2454	1227	680	378	220

Correlation Coefficient	Slope	Y Intercept
-1.000 to -0.980	-0.400 to -0.100	2,500 to 3,500
-0.999	-0.269	2,855
-0.998	-0.261	2,848

図15 : ENH29A

Performed by: SUPERVISOR LAL Lot No.: YL039E72VQ Exp: 4/20/2024 Time: 1:20:54PM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: ENH29A Endotoxin Lot No.: ENH29A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.999 Slope = -0.269 Y Intercept = 2,855

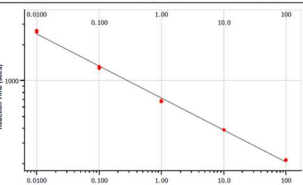
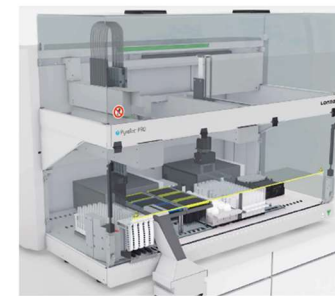
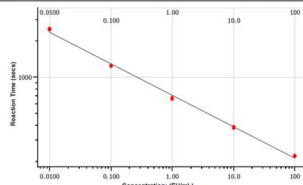


図16 : N-ENH01A

Performed by: SUPERVISOR LAL Lot No.: YL039E72VQ Exp: 4/20/2024 Time: 2:46:59PM (UTC+9)
 Method: PYROCENT-5000 - Initial Qualification Water Lot No.: 000109068 Exp: 3/28/2023 Date: 4/1/2024
 Test: N-ENH01A Endotoxin Lot No.: N-ENH01A Exp: 4/1/2024 Summe
 Temperature: 36.0°C - 37.3°C Reconc. Buffer Lot No.: 000110611 Exp: 4/1/2024 S/N: 200207593
 Linear Regression: Correlation Coefficient = -0.998 Slope = -0.261 Y Intercept = 2,848



8. 蛍光法（欧州薬局方 Chapter2.6.32 収載）：PyroGene™ r Fc

検量線濃度；0.005-0.05-0.5-5.0 EU/mL（4 濃度）

結果：表 7,8、図 17,18

① 日本薬局方エンドトキシン標準品 従来ロット

日本薬局方エンドトキシン標準品：ENH29A					Correlation Coefficient	Slope	Y Intercept	
使用ライセート試薬：PyroGene（蛍光法rFC）								
表 7	LOT	0.005	0.05	0.5	5	0.980 to 1.000	0.760 to 1.110	2.500 to 5.000
ZL042A7VJP		180	1707	14444	66640	0.996	0.860	4.306
		177	1729	14315	65743			
		191	1705	14365	64904			



② 日本薬局方エンドトキシン標準品 新ロット

日本薬局方エンドトキシン標準品：N-ENH01A					Correlation Coefficient	Slope	Y Intercept	
使用ライセート試薬：PyroGene（蛍光法rFC）								
表 8	LOT	0.01	0.1	1	10	0.980 to 1.000	0.760 to 1.110	2.500 to 5.000
ZL042A7VJP		142	1532	12573	60556	0.996	0.880	4.264
		148	1573	12889	61111			
		140	1536	13031	60178			

図17：ENH29A

Performed By: SUPERVISOR	rFC Enzyme Solution Lot No.: ZL042A7VJP	Exp: 11/10/2024	Time: 9:53:09AM (UTC+9)
PyroGene - Initial Qualification Test	Water Lot No.: 0001209538	Exp: 3/29/2025	Date: 4/2/2024
Template: JP-RSE ENH29A x	Endotoxin Lot No.: ENH29A	Exp: 4/4/2024	PyroGene018
ZL042A7VJP	rFC Assay Buffer Lot No.: 0001212566	Exp: 11/24/2024	S/N 14110429
Temperature: 36.9°C - 37.0°C	Fluorogenic Substrate Lot No.: 0001212570	Exp: 11/17/2024	
Linear Regression: Correlation Coefficient = 0.996	Slope = 0.860	Y-Intercept = 4.306	

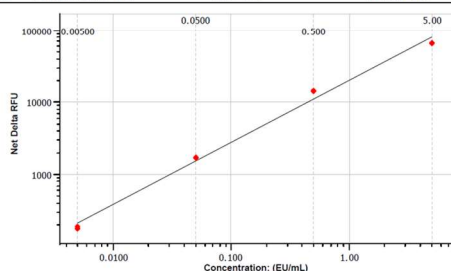
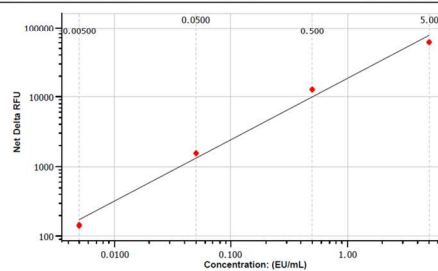


図18：N-ENH01A

Performed By: SUPERVISOR	rFC Enzyme Solution Lot No.: ZL042A7VJP	Exp: 11/10/2024	Time: 9:53:09AM (UTC+9)
PyroGene - Initial Qualification Test	Water Lot No.: 0001209538	Exp: 3/29/2025	Date: 4/2/2024
Template: JP-RSE N-ENH01A x	Endotoxin Lot No.: N-ENH01A	Exp: 4/18/2024	PyroGene018
ZL042A7VJP	rFC Assay Buffer Lot No.: 0001212566	Exp: 11/24/2024	S/N 14110429
Temperature: 36.9°C - 37.0°C	Fluorogenic Substrate Lot No.: 0001212570	Exp: 11/17/2024	
Linear Regression: Correlation Coefficient = 0.996	Slope = 0.880	Y-Intercept = 4.264	



日本薬局方エンドトキシン標準品の現行製品（Lot ENH29A）および新製品（Lot N-ENH01A）での
 用手法による操作、エンドトキシン自動測定システム（PyroTecPro™）による操作のいずれにおいても、

1. 用量反応直線に差がなく、いずれも相関係数 $|r| \geq 0.980$ を満たし、相関係数 $|r|$ は、1.000~0.995 となり、非常に良好な相関係数を示した。
2. 各検量線の「Slope、Y Intercept」も LONZA 社 エンドトキシン検出ソフトウェア WinKQCL™で推奨している規定範囲内に、いずれも収まった。
3. また、各検量線濃度における CV%も各ライセート試薬において、LONZA 社推奨範囲内である <10%であった。

以上の結果から、日本薬局方エンドトキシン標準品の現行製品（Lot ENH29A）および新製品（Lot N-ENH01A）での有意な差は認められず、また、いずれの操作方法においても同等の結果を示した。

以上