CERTIFICATION

AOAC Research Institute

*Performance Tested Methods*SM

Certificate No.

051901

The AOAC Research Institute hereby certifies the method known as:

LuciPac A3 Surface

manufactured by

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 1005-0003
Japan

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods* SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates, Senior Director
Signature for AOAC Research Institute

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METHOD NAME
LuciPac A3 Surface

CATALOG NUMBER
60361

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APPLICABILITY OF METHOD
Analytes – Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP)

Matrixes – Stainless steel

Performance claims – According to the linear regression and other statistical approaches, the LuciPac A3 Surface for Hygiene Monitoring is effective at detecting the presence of total adenylate (ATP+ADP+AMP) on stainless steel surfaces in food processing and food service facilities with an LOD of 3.3 fmol ATP, 0.9 fmol ADP and 1.8 fmol AMP.

ORIGINAL CERTIFICATION DATE
May 22, 2019

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

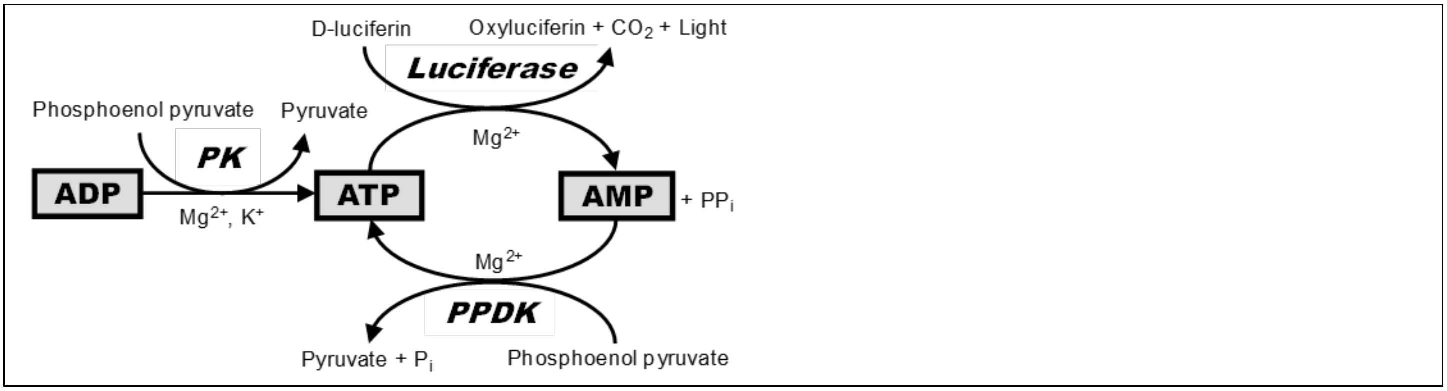
METHOD MODIFICATION RECORD
1. **November 2019 Level 2**

SUMMARY OF MODIFICATION
1. **Addition of Lumitester Smart luminometer.**

Under this AOAC *Performance Tested Methods*[SM] License Number, 051901 this method is distributed by:
1. **AS ONE CORPORATION**
2. **FUJIFILM Wako Pure Chemical Corporation**
3. **KENIS LIMITED**
4. **Nippon Bacterial Test Co., Ltd.**
5. **Weber Scientific**

Under this AOAC *Performance Tested Methods*[SM] License Number, 051901 this method is distributed as:
1. **LuciPac A3 Surface**
2. **LuciPac A3 Surface**
3. **LuciPac A3 Surface**
4. **LuciPac A3 Surface**
5. **LuciPac A3 Surface**

PRINCIPLE OF THE METHOD (1)
The principle of detection of A3 is shown in Figure 1. Firefly luciferase can produce light in the presence of ATP, luciferin, oxygen and Mg^{2+}. The amount of light produced is proportional to the amount of ATP in a sample and therefore ATP can be quantified by measuring the light produced through this reaction using a luminometer, showing a reading of Relative Light Units (RLUs). This is well known as the ATP method. In order to detect AMP simultaneously and maintain the light production, ATP was regenerated from AMP using pyruvate orthophosphate dikinase reactions (PPDK) in the presence of phosphoenol pyruvate, inorganic pyrophosphate (PPi) and Mg^{2+} (Figure 1). Furthermore, ADP is converted to ATP by pyruvate kinase (PK, Figure 1). This allows the test to detect and quantify total adenylate and dramatically increases the signal available to the test.



DISCUSSION OF THE VALIDATION STUDY (1)

ATP tests are commonly used for an assessment of hygienic conditions in food industry. It should be noted that adenylate swabbing assays including ATP and the A3 test are not for microorganism detection but for cleaning verification because adenylates are not specific to microorganisms as shown in Table 3 and 4. However, monitoring the surface after cleaning is effective for preventing foodborne illness for the following reasons. First, food residues on surfaces are the source of nutrients for microorganisms. Second, organic matter can interfere with the antimicrobial activity of disinfectants (5) and decrease sanitation efficiency. Moreover, cleaning verification also seems to be effective for preventing food allergen cross-contact that can occur via the transfer of allergens in the same facility or on the same processing line for the allergen-containing and nonallergen-containing foods or ingredients.

A validation study of a conventional ATP monitoring test on stainless steel surfaces has been reported (3). Recently, the LuciPac A3 Surface Hygiene Monitoring System that can detect ATP+ADP+AMP (A3) has been developed and it shows more advanced sensitivity to determine food/organic debris compared to the conventional ATP tests (2). However, there is no report about the method validation for A3 assay. Here we report the validation study of the LuciPac A3 Surface Hygiene Monitoring System under the specific guidelines of the AOAC Research Institute *Performance Tested Method*SM program.

Firstly, pure analyte assays were performed to determine the LODs of ATP, ADP and AMP. The results in the method developer laboratory and the independent laboratory were consistent (Table 2). The LODs were around 10 RLU. According to the regression analyses, LODs can be expressed as ca. 2.5 fmol/assay on a molecular basis. RSD_r values <20% were achieved at or above 2.5 fmol, though RSD_r values of analyte-free water and 1.0 fmol adenylate were 20-60% (Table 1). This study also demonstrated good linearity of detection sensitivity [$R^2 > 0.9862$].

In order to determine the feasibility of detecting food matrix residues on stainless steel surfaces, the surface was treated with dilutions of 5 food matrices, i.e. raw poultry (raw chicken breast), ready to eat meat product (sliced deli ham), fresh produce and Juice (orange juice), heat processed milk and dairy (yogurt) and chocolate/bakery products (apple pie). All matrices showed sufficient reactivity as reported previously and a response that varied with dilution (Table 3). Method Developer Studies demonstrated that pure analyte solutions yielded <20% RSD_r (Table 1), but RSD_r values of each matrix solution for swabbing assays were <30%. Independent laboratory Studies demonstrated that RSD_r values of each matrix solution for swabbing assays were <26.7% (orange juice) and <42.5% (ham, Table 3). The higher variations of matrixes were likely caused by additional factor, i.e. swabbing technique. Additionally, regarding insoluble food samples, solid and liquid are separated soon even after careful homogenization. This unavoidable heterogeneity may cause variability in the amount of matrix applied onto the plates. It should also be considered that all cotton swabs may not be able to pick up the dried solid particles completely. Consistent swabbing technique is important to minimize the variability. Swabbing an object thoroughly using the entire surface of the swab with rotation is ideal. Ideally the swab should be slightly bent when exerting appropriate pressing force.

Three pure cultures of microorganisms, a Gram-negative bacterium (*C. sakazakii*), a Gram-positive bacterium (*L. acidophilus*), and a yeast species (*S. cerevisiae*) were also tested using stainless steel surfaces. As is the case with food matrices, RLU responses to the organism concentration were observed (Table 4). RSD_r values of each microbial solution for swabbing assays (10-35%) were also comparable to the food matrix study. Consequently, validation study using stainless steel surface demonstrated that the LuciPac A3 Surface Hygiene Monitoring System provides rapid and precise food/organic debris determination.

Disinfectants are used in cleaning to kill microorganisms, and these chemicals may be left on the surface. According to our previous study, sodium hypochlorite (500 ppm), ethanol (80%) and quaternary ammonium (benzalkonium chloride, 0.1%) inhibit the A3 assays to some extent (ca. 10% inhibition) when 10 μL of disinfectants were added to the moistened swab (2). In this study, inhibition effects were evaluated using the stainless steel surface model to closely mimic industrial cleaning practices (Table 5 and 6). Since ethanol can be completely evaporated, another sanitizer for food processing, peracetic acid (6%), was tested instead in this study. Similar to our previous result, sodium hypochlorite did not affect the result significantly under these conditions. Quaternary ammonium inhibited 25-30% of the ATP signal. Contrary to our expectations, peracetic acid amplified the RLU output. Acid compounds generally reduce RLU values due to lowering pH of the reaction mixture from the optimum. The reason of the enhancement by peracetic acid on stainless steel is unclear. The peracetic acid that was used in this study is composed of hydrogen peroxide, acetic acid, buffer, chelator and stabilizer based on the manufacturer's information. Peracetic acid (boiling point: 105°C) and acetic acid (boiling point: 118°C) seem to have been completely evaporated and other components might enhance the measurement values. As described above, the LuciPac A3 Surface hygiene monitoring system is intended for cleaning verification. Moreover, Table 5 and 6 indicate that it may be affected by chemical agents. Therefore, the A3 test is recommended to be used after rinsing away sanitizing agents for accurate assessment.

Table 1. Method developer and independent laboratory pure analyte results using LuciPac A3 Surface/Lumitester PD-30 system. (A) Adenosine triphosphate (ATP), (B) Adenosine diphosphate (ADP) and (C) Adenosine monophosphate (AMP) (1)

(A)

		ATP, fmol/assay						
		0	1	2.5	5	10	25	100
Method developer	Mean RLU[a]	5.2	7.0	10.3	14.1	22.2	46.0	179.4
	s_r[b]	1.6	2.0	1.3	1.9	2.3	3.9	8.7
	RSD_r, %[c]	31.1	28.6	13.0	13.1	10.1	8.5	4.8
	Mean fmol[d]	0.1	1.2	3.1	5.3	9.9	23.6	100.3
Independet laboratory	Mean RLU	3.1	5.5	9.9	13.2	21.4	41.7	163.7
	s_r	1.7	2.9	1.7	1.3	2.4	4.3	9.9
	RSD_r, %	55.8	53.0	16.8	10.0	11.3	10.3	6.0
	Mean fmol	-0.8	0.7	3.5	5.6	10.7	23.5	100.3

(B)

		ADP, fmol/assay						
		0	1	2.5	5	10	25	100
Method developer	Mean RLU	4.9	7.0	9.3	14.0	23.0	52.4	178.2
	s_r	1.4	1.6	1.3	2.6	1.8	3.7	17.9
	RSD_r, %	28.0	23.3	13.5	18.7	7.7	7.2	10.0
	Mean fmol	-0.5	0.7	2.1	4.8	10.0	26.9	99.5
Independet laboratory	Mean RLU	4.0	7.0	9.9	15.0	21.6	48.3	187.5
	s_r	1.5	1.6	1.0	2.1	2.8	4.8	8.7
	RSD_r, %	37.3	22.3	10.0	13.7	13.1	10.0	4.6
	Mean fmol	-0.3	1.4	3.0	5.8	9.4	24.0	100.3

(C)

		AMP, fmol/assay						
		0	1	2.5	5	10	25	100
Method developer	Mean RLU	6.5	8.6	10.0	16.7	24.8	52.8	195.6
	s_r	1.2	2.1	1.3	1.4	2.9	3.4	15.8
	RSD_r, %	18.1	24.0	13.3	8.5	11.7	6.5	8.1
	Mean fmol	0.2	1.3	2.0	5.5	9.8	24.6	100.1
Independet laboratory	Mean RLU	5.3	7.9	9.3	13.8	20.9	48.8	180.9
	s_r	1.3	1.9	1.4	2.3	2.2	2.0	11.3
	RSD_r, %	25.2	24.2	15.2	17.0	10.7	4.1	6.3
	Mean fmol	0.2	1.7	2.5	5.0	9.1	24.9	100.1

[a] Relative Light Unit. Ten replicates were tested at each concentration.

[b] Standard Deviation of Repeatability.

[c] Relative Standard Deviation of Repeatability.

[d] Amounts of the adenylate were converted from the mean RLU values using the linearity curves in Figure 2 (Method developer) and 4 (Independent laboratory).

Table 2. Estimation of limit of detection (LOD) for adenosine triphosphate (ATP), adenosine diphosphate (ADP), and (C) adenosine monophosphate (AMP)from the method developer and independent laboratory data of pure analytes using LuciPac A3 Surface/Lumitester PD-30 system. (1)

	Adenylate	$\bar{X}_o$ [a]	s_b [b]	m [c]	Calculated LOD,	
					RLU[d]	fmol/assay[e]
Method developer	ATP	5.2	1.4292	0.0409	10.6	3.3
	ADP	4.9	0.3848	0.0955	7.3	0.9
	AMP	6.5	0.5710	0.0767	9.6	1.8
Independent laboratory	ATP	3.1	1.5732	0.0511	9.1	3.0
	ADP	4.0	1.5339	0.0400	9.7	2.9
	AMP	5.3	0.9548	0.0554	9.3	2.5

[a] The mean analytical value of the known negative matrix (Mean RLU for 0 fmol/assay in Table 1).

[b] The intercept of the plots of standard deviation vs. mean LuciPac A3 Surface responses (Figure 3).

[c] The slope of the plots of standard deviation vs. mean LuciPac A3 Surface responses (Figure 3).

[d] Relative Light Unit. Each LOD (RLU) were calcurated using the formula: $(\bar{X}_o + 3.3 \text{ x } s_b)/(1–1.65\,m)$

[e] Each LOD (fmol/assay) was calculated by LOD (RLU) using the linearity curves in Figure 2 (Method developer) and 4 (Independent laboratory).

Table 3. Replicate Relative Light Unit (RLU), mean RLU, s_r and RSD_r of the LuciPac A3 Surface method determined with various matrixes (1)

Matrix	Target RLU	Dilution factor	Replicate RLU 1	2	3	4	5	6	7	8	9	10	Mean RLU	s_r[c]	RSD_r, %[d]
Raw chicken breast[a]	1000-500	1000	823	865	919	829	739	790	958	892	655	795	827	89	11
	500-200	5000	396	172	192	364	216	222	232	307	293	371	277	81	29
	200-75	10000	126	136	144	132	116	112	101	140	86	143	124	19	16
	<75	30000	55	43	64	47	62	49	62	79	98	50	61	17	28
	Background		14	12	13	21	27	9	26	30	21	25	20	7	37
Sliced deli ham[a]	1000-500	10000	686	533	734	698	710	1163	1075	1098	1018	944	866	218	25
	500-200	33000	262	182	282	194	294	270	343	380	392	347	295	72	24
	200-75	100000	128	93	102	112	129	115	135	128	148	107	120	17	14
	<75	330000	95	93	58	48	57	58	69	69	57	55	66	16	24
	Background		13	15	14	14	12	29[e]	18	13	15	14	14	2	12
Orange juice[a]	1000-500	5000	556	846	865	672	769	960	986	749	668	617	769	144	19
	500-200	10000	193	284	239	241	193	266	208	236	252	324	244	41	17
	200-75	30000	115	75	84	76	65	85	121	107	90	73	89	19	21
	<75	100000	25[e]	47	47	54	49	47	36	54	49	46	48	5	11
	Background		18	20	25	20	27	29	25	11	13	15	20	6	30
Yogurt[a]	1000-500	2000	857	811	902	940	1004	806	1068	807	980	906	908	90	10
	500-200	5000	386	313	306	304	294	468	642	559	523	364	416	124	30
	200-75	16000	124	119	104	181	86	108	172	106	111	115	123	30	25
	<75	32000	43	66	66	55	55	51	76	40	59	47	56	11	20
	Background		23	9	18	16	11	17	12	20	18	15	16	4	27
Apple pie[a]	1000-500	300	586	709	647	668	646	714	623	621	631	765	661	54	8
	500-200	500	348	424	333	329	376	414	325	314	352	343	356	37	11
	200-75	3000	67	89	74	101	107	112	77	81	107	98	91	16	17
	<75	5000	31	42	35	41	40	37	31	38	51	48	39	7	17
	Background		19	14	18	15	16	14	19	13	23	11	16	4	22
Sliced deli ham[b]	1000-500	60000	785	543	395	465	1011[e]	461	620	534	571	503	542	113	20.8
	500-200	80000	290	200	134	182	279	278	148	411	223	160	231	85	36.9
	200-75	100000	399[e]	118	173	167	108	186	88	173	105	142	140	36	25.8
	200-75	120000	172	183	403[e]	281	124	170	101	124	80	90	147	63	42.5
	200-75	160000	173	304[e]	114	114	78	70	111	61	57	130	101	38	37.6
	<75	400000	153[e]	50	39	43	45	66	45	48	55	46	49	8	16.3
	Background		39	42	34	34	37	42	37	28	40	37	37	4	11.5
Orange juice[b]	1000-500	4000	692	686	516	596	712	721	631	648	661	474	634	83	13.1
	500-200	8000	160	219	202	203	239	240	177	208	189	273	211	33	15.7
	200-75	10000	135	137	144	196	142	195	171	152	148	248	167	36	21.8
	200-75	12000	121	244	160	125	225	157	187	215	141	168	174	42	24.3
	<75	40000	90	47	55	48	46	46	53	49	56	79	57	15	26.7
	Background		41	40	38	40	40	39	31[e]	40	38	43	40	2	3.9

[a] Method developer study.
[b] Independent laboratory study.
[c] Standard Deviation of Repeatability.
[d] Relative Standard Deviation of Repeatability.
[e] Excluded from data analysis based on Grubbs' test.

Table 4. Replicate Relative Light Unit (RLU), mean RLU, s_r and RSD_r of the LuciPac A3 Surface method determined with various microbes (1)

Organism	Target RLU	Theoretical cfu/ml[a]	Replicate RLU 1	2	3	4	5	6	7	8	9	10	Mean RLU	s_r[b]	RSD_r, %[c]
C. sakazaki	1000-500	2.0×10^6	730	574	576	675	604	600	644	534	634	536	611	62	10
	500-200	8.6×10^5	284	377	293	292	310	278	252	330	329	266	301	37	12
	200-75	3.0×10^5	146	93	103	108	139	127	108	144	123	139	123	19	15
	<75	1.5×10^5	87	65	71	48	34	31	67	86	61	56	61	19	31
	Background	0	17	16	35	28	13	14	36	20	19	17	22	8	39
L. acidophilus	1000-500	2.0×10^5	1258	907	585	660	1081	1086	648	791	674	776	847	227	27
	500-200	4.3×10^4	223	230	248	229	320	222	209	254	287	267	249	34	14
	200-75	2.0×10^4	64	74	56	82	146	53	63	103	113	85	84	29	35
	<75	1.0×10^4	34	41	39	64	39	40	44	49	42	52	44	9	19
	Background	0	10	9	11	12	31	25	8	9	11	15	14	8	55
S. cerevisiae	1000-500	6.7×10^3	989	1139	818	887	1117	912	926	926	1104	1114	993	116	12
	500-200	2.0×10^3	289	298	296	281	226	372	204	256	280	195	270	52	19
	200-75	6.7×10^2	143	131	71	152	98	110	67	51	86	80	99	34	35
	<75	3.3×10^2	42	31	25	39	33	27	26	18	22	27	29	7	25
	Background	0	11	8	11	33[d]	13	11	17	22	20	13	14	5	33

[a] Each value was obtained by deviding the colony forming unit of each undiluted suspention by dilution factors. The actual amount of organism added to the coupon was 250 µL.

[b] Standard Deviation of Repeatability.

[c] Relative Standard Deviation of Repeatability.

[d] Excluded from data analysis based on Grubbs' test.

Table 5. Replicate Relative Light Unit (RLU) and mean RLU for the effect of common sanitizers on the LuciPac A3 Surface method (1)

	Replicate RLU																	
	Water						1000 fmol ATP[a]						4000 fmol ATP					
Sanitizer	1	2	3	4	5	Mean	1	2	3	4	5	Mean	1	2	3	4	5	Mean
None (Water)	23	17	21	15	22	20	147	126	128	160	144	141	417	611	394	589	330	468
Sodium Hypochlorite	31	32	22	30	32	29	148	127	180	168	180	161	382	534	539	506	611	514
Peracetic acid	46	59	83	58	77	65	237	415	208	322	276	292	1239	1343	1235	1352	984	1231
Quaternary ammonium	24	30	28	30	22	27	145	134	110	109	150	130	334	451	282	422	327	363

[a] Adenosine triphosphate

Table 6. Effect of common sanitizers on the LuciPac A3 Surface method (1)

	Mean RLU[a]							
	Water		1000 fmol ATP[b]		4000 fmol ATP		Inhibition, %[c]	
Sanitizer	C[d]	S[e]	CA[f]	SA[g]	CA	SA	1000 fmol ATP	4000 fmol ATP
Sodium Hypochlorite	20	31	141	161	468	514	-8	-8
Peracetic acid	20	65	141	292	468	1231	-187	-160
Quaternary ammonium	20	27	141	130	468	363	29	25

[a] Relative Light Unit

[b] Adenosine triphosphate

[c] A negative percent inhibition correlated to an increase in signal. Calculated using mean RLU and the following equation: Inhibition (%) = {1-[(SA-S)/(CA-C)]}x100.

[d] C = Signal from the control (analyte-free water) on the control surface (analyte-free water dried onto the stainless steel surface).

[e] S = Signal from the control (analyte-free water) on the disinfectant surface (disinfectant dried onto the stainless steel surface).

[f] CA = Signal from ATP on the control surface (analyte-free water and ATP dried onto the stainless steel surface).

[g] SA = Signal from ATP on the disinfectant surface (disinfectant and ATP dried onto the stainless steel surface).

DISCUSSION OF THE MODIFICATION APPROVED NOVEMBER 2019 (7)

In the first validation study for the LuciPac A3/the Lumitester PD-30 Hygiene Monitoring System for the detection of ATP, ADP, and AMP from stainless steel surfaces, pure analyte solutions, detection of food residues and microbial residues on stainless steel surfaces, interference by disinfectants, selectivity of the method response, instrument variation, lot-to-lot consistency, and accelerated stability were evaluated. In this modification validation study for the new instruments, Lumitester Smart, pure analyte study and instrument variation were carried out in order to evaluate whether the ability of Lumitester Smart to detect pure ATP, ADP, and AMP was comparable with that of Lumitester PD-30. Detection of food residues and microbial residues on stainless steel surfaces, interference by disinfectants, selectivity of the method response, instrument variation, lot-to-lot consistency, and accelerated stability are accordingly ensured by the previous validation data because these factors depend on the performances of the swab.

The LODs for ATP, ADP, and AMP were 1.6, 3.5, and 3.0 fmol/assay, respectively (Table 2). Pure ATP, ADP, and AMP were detected by the LuciPac A3 Surface/Lumitester Smart system with good linearity (R^2 > 0.9866) (Figure 2), and repeatability precision (RSD_r: 9.6-18.9 % for 1-100 fmol ATP/assay, 6.4-16.5 % for 2.5-100 fmol ADP/assay, 6.1-15.5 % for 2.5-100 fmol AMP/assay) (Table 1). In our previous report of pure analyte studies using LuciPac A3 Surface/Lumitester PD-30 system (AOAC *Performance Tested Method*SM 051901), the LODs for ATP, ADP, and AMP were 3.0-3.3, 0.9-2.9, 1.8-2.5 fmol/assay, respectively. The repeatability precision (RSD_r) of the measurements were 4.8-16.8 % for 2.5-100 fmol ATP assay, 4.6-23.3 % for 1-100 fmol ADP/assay, and 4.1-24.2 % for 1-100 fmol AMP assay. The linearity (R^2) of the measures were 0.9862 or higher.

In the instrument variation studies, no significant difference could be found at any ATP concentration among the three Lumitester Smart (Table 3).

These results indicated that the performance of LuciPac A3 Surface/Lumitester Smart system to detect pure ATP, ADP, and AMP was comparable with that of LuciPac A3 Surface/Lumitester PD-30 system.

Table 1. Pure analyte results using LuciPac A3 Surface/Lumitester Smart system. (A) Adenosine triphosphate (ATP), (B) Adenosine diphosphate (ADP) and (C) Adenosine monophosphate (AMP) (7)

A

	ATP, fmol/assay						
	0	1	2.5	5	10	25	100
Mean RLU[a]	7.6	9.7	14.9	17.1	26.2	53.1	173.6
s_r[b]	1.4	1.8	2.5	1.9	3.3	5.2	16.7
RSD_r, %[c]	18.8	18.9	16.9	11.2	12.4	9.9	9.6
Mean fmol[d]	-1.1	0.2	3.4	4.7	10.2	26.5	99.6

B

	ADP, fmol/assay						
	0	1	2.5	5	10	25	100
Mean RLU	6.7	10.7	11.9	15.9	25.0	50.7	181.9
s_r	2.2	2.5	2.0	2.3	3.3	3.8	11.6
RSD_r, %	33.0	22.9	16.5	14.7	13.2	7.5	6.4
Mean fmol	-0.5	1.8	2.5	4.8	10.0	24.8	100.1

C

	AMP, fmol/assay						
	0	1	2.5	5	10	25	100
Mean RLU	7.3	10.9	13.2	16.0	25.8	51.0	181.6
s_r	2.3	2.9	2.0	1.5	3.3	3.6	11.1
RSD_r, %	31.0	26.5	15.5	9.3	12.9	7.0	6.1
Mean fmol	-0.5	1.6	2.9	4.5	10.2	24.7	100.1

[a] Relative Light Unit. Ten replicates were tested at each concentration.

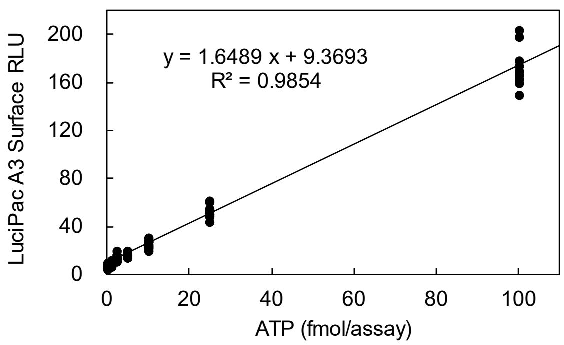
[b] Standard Deviation of Repeatability.

[c] Relative Standard Deviation of Repeatability.

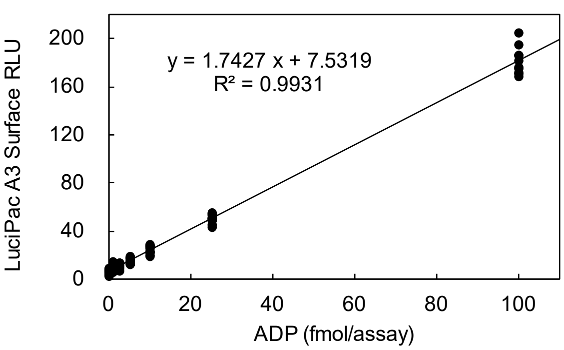
[d] Amounts of the adenylate were converted from the mean RLU values using the linearity curves in Figure 2.

Figure 2. Dose response curves. LuciPac A3 Surface Relative Light Unit (RLU) responses for (A) adenosine triphosphate (ATP); (B) adenosine diphosphate (ADP); and (C) adenosine monophosphate (AMP). (7)

A



B



C

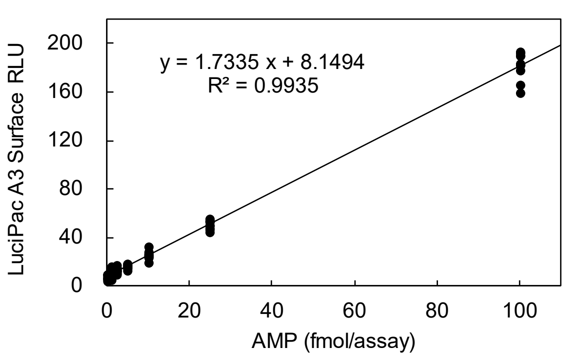


Table 2. Estimation of limit of detection (LOD) for adenosine triphosphate (ATP), adenosine diphosphate (ADP), and (C) adenosine monophosphate (AMP)from the data of pure analytes using LuciPac A3 Surface/Lumitester Smart system. (7)

Adenylate	$\bar{X}_0$[a]	s_b[b]	m[c]	Calculated LOD, RLU[d]	Calculated LOD, fmol/assay[e]
ATP	7.6	0.7497	0.0916	11.9	1.5
ADP	6.7	1.6060	0.0541	13.2	3.2
AMP	7.3	1.5531	0.0517	13.6	3.1

[a] The mean analytical value of the known negative matrix (Mean RLU for 0 fmol/assay in Table 1).
[b] The intercept of the plots of standard deviation vs. mean LuciPac A3 Surface responses (Figure 3).
[c] The slope of the plots of standard deviation vs. mean LuciPac A3 Surface responses (Figure 3).
[d] Relative Light Unit. Each LOD (RLU) were calcurated using the formula: $(\bar{X}_0 + 3.3 \text{ x } s_b)/(1–1.65\ m)$.
[e] Each LOD (fmol/assay) was calculated by LOD (RLU) using the linearity curves in Figure 2.

Table 3. Results of Instrument variation study (7)

ATP[a], fmol	Replicate	23°C 1[d]	23°C 2[d]	23°C 3[d]	10°C 1[d]	10°C 2[d]	10°C 3[d]
0	1	6	8	7	7	6	5
	2	6	8	7	8	7	7
	3	9	7	7	5	6	8
	4	6	5	6	4	6	7
	5	4	4	5	8	9	8
	Mean	6.2	6.4	6.4	6.4	6.8	7.0
	s_r[b]	1.8	1.8	0.9	1.8	1.3	1.2
	RSD_r, %[c]	28.9	28.4	14.0	28.4	19.2	17.5
50	1	96	96	106	122	110	103
	2	99	96	110	113	109	97
	3	104	95	90	102	105	100
	4	90	93	106	106	112	119
	5	94	98	95	108	114	115
	Mean	96.6	95.6	101.4	110.2	110.0	106.8
	s_r	5.3	1.8	8.5	7.7	3.4	9.7
	RSD_r, %	5.5	1.9	8.4	7.0	3.1	9.0
500	1	978	943	964	1233	931	1203
	2	994	958	992	1092	1208	1085
	3	941	911	927	1141	1012	1009
	4	964	996	887	980	1098	1170
	5	927	881	878	999	1075	1002
	Mean	960.8	937.8	929.6	1089.0	1064.8	1093.8
	s_r	27.1	44.1	48.9	104.2	103.0	91.4
	RSD_r, %	2.8	4.7	5.3	9.6	9.7	8.4

Column values are Relative Light Unit (RLU).

[a] Adenosine triphosphate.
[b] Standard Deviation of Repeatability.
[c] Relative Standard Deviation of Repeatability.
[d] Serial No. 1: 1911053130070S, 2: 1849053130043S, 3: 1902053130100S.

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