CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

112201

The AOAC Research Institute hereby certifies the method known as:

LuciPac A3 Surface (Pre-Moistened)

manufactured by

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi
Minato-ku, Tokyo 105-0003
Japan

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. 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.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

Issue Date November 20, 2024
Expiration Date December 31, 2025

METHOD NAME	CATALOG NUMBER	ORIGINAL CERTIFICATION DATE
LuciPac A3 Surface (Pre-Moistened)	60367	November 07, 2022

PRINCIPLE OF THE METHOD

The principle of detection of A3 is shown in Figure 1 (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 is regenerated from AMP using pyruvate orthophosphate dikinase reaction (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. Both Lumitester PD-30 and Lumitester Smart detect the light with a photodiode.

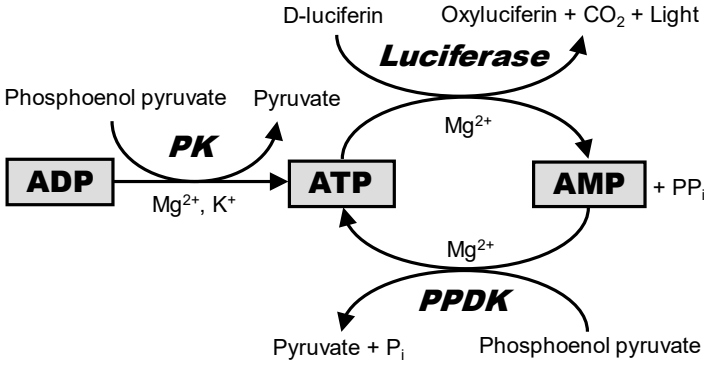

Figure 1. The principle of ATP+ADP+AMP (A3) detection. Adenosine triphosphate (ATP) can be quantified by the detection of the light from the firefly luciferase reaction. Adenosine monophosphate (AMP) is recycled to ATP using the pyruvate orthophosphate dikinase (PPDK). Adenosine diphosphate (ADP) is converted to ATP by the pyruvate kinase reaction (PK). PPi : inorganic pyrophosphate, Pi : inorganic phosphate.

CERTIFIED CLAIM STATEMENT: The LuciPac A3 Surface (Pre-Moistened) method is certified for the determination of ATP+ADP+AMP within the scope of Tables 1 and 2.

***Certification includes**:*

1. Lumitester Smart (Cat. No. 61234) or Lumitester PD-30 (Cat. No. 60486) reader with optional temperature compensation mode.
2. Lumitester Control Kit (Cat. No. 60484) for calibration of reader.

Table 1. Method Performance Claims

			Performance Supporting Certification		
Surface	Analyte or Material	Range tested[b]	Mean RLU range	LOD, fmol/assay	RSD_r, %
None	ATP	1–100 fmol/assay	8.6–216	7.0	6–61
	ATP[a]	1–100 fmol/assay	11.8–212	3.1	11–23
	ADP	1–100 fmol/assay	8.5–219	4.3	6–43
	ADP[a]	1–100 fmol/assay	13.5–218	3.7	7–30
	AMP	1–100 fmol/assay	7.7–239	5.2	6–59
	AMP[a]	1–100 fmol/assay	11.1–235	4.9	7–22
Stainless Steel (10 cm x 10 cm)	Raw chicken breast	1000–10,000 df	61–811	N/A[c]	6–17
	Sliced deli ham	70,000–800,000 df	37–711	N/A	21–44
	Sliced deli ham[a]	70,000–1,600,000 df	39–842	N/A	8–21
	Orange juice	4000–64,000 df	41–619	N/A	27–39
	Orange juice[a]	4000–100,000 df	22–743	N/A	17–26
	Yogurt	1200–12,000 df	41–808	N/A	17–25
	Apple pie	1000–8000 df	71–502	N/A	20–52
	Cronobacter sakazakii	1.8–8.5 x 10^4 cfu	72–510	N/A	6–13
	Lactobacillus acidophilus	0.6–12 x 10^3 cfu	60–750	N/A	9–10
	Saccharomyces cerevesiae	40–1450 cfu	49–822	N/A	6–26

[a] Tested by the Independent Laboratory.

[b] df = dilution factor; cfu = colony forming unit.

[c] N/A = Not applicable.

Table 2. Method Selectivity

		No. Compounds Interfering[a]	
Compounds	Concentration	0 fmol ATP, ADP, AMP	100 fmol ATP, ADP, AMP
10 Non-target adenylates	2500 fmol	0	0
		1000 fmol ATP	4000 fmol ATP
3 Disinfectants	Residual	0	0

[a] Interference defined as an effect ≥20%.

Table 3. Summary

No.	Date	Summary	Supporting Data
1	November 2022	Level 2 Modification: PTM 051901 modified with a pre-moistened swab and liquid reagents for a new certification PTM 112201.	Certification Report
2	May 2024	Level 3 Modification: Modified luciferase clone; optimized reagents; new pouch material.	Modification Report 1