

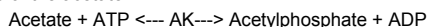
Reagent for enzymatic determination of acetic acid (Acetate) in foodstuff and other sample material, on automated photometric systems

Method

The method is based on Acetate kinase (AK).

Principle

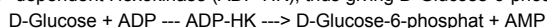
Acetic acid (Acetate) and Adenosine-5'-triphosphate (ATP) react in presence of the enzyme Acetate kinase (AK), leading to Acetylphosphate and Adenosin-5'-diphosphat (ADP). The equilibrium of this reaction is on the left side of the acetate:



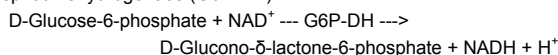
In order to displace the reaction to the right, the Acetylphosphate is transformed into Acetyl-CoA plus phosphate by the combination of Coenzyme A (CoA) and Phosphotransacetylase (PTA):



The ADP produced in the first reaction reacts with D-Glucose through ADP-dependent Hexokinase (ADP-HK), thus giving D-Glucose-6-phosphate:



This reaction is followed by a second one involving the enzyme Glucose-6-phosphat Dehydrogenase (G6P-DH):



During this reaction, Nicotinamid-adenin-dinucleotid (NAD) is reduced to NADH. The NADH-concentration is proportional to the acetic acid concentration.

Storage instructions and reagent stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2 – 8 °C and if contamination is avoided. Do not freeze!

Warnings and precautions

1. The reagents contain sodium azide (0.95 g/l) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. Take the necessary precautions for the use of laboratory reagents.

Reagent preparation

The reagents and the standard are ready-to-use.

Materials required but not provided

Distilled water (aseptic and free of heavy metal) and general laboratory equipment.

Package content and concentration of the reagents

R1	4 x 20 mL	Buffer	pH 8
		D-Glucose	10 mmol/L
		AK	≥ 2 kU/L
		PTA	≥ 6 kU/L
		ADP-HK	≥ 300 U/L
		G6PDH	≥ 4 kU/L
R2	4 x 5 mL	Buffer	pH 6,5
		NAD	≥ 15 mmol/L
		ATP	≥ 5 mmol/L
		CoA	≥ 0.3 mmol/L

Sample preparation

If the sample has one of the characteristics below, which might hamper the test, please follow the corresponding sample preparation procedure:

- Use clear, colourless and practically neutral liquid samples directly, or after dilution to an Acetic acid concentration up to 1200 mg/L.
- Use wine and fruit juice samples directly in the assay, provided they contain now turbidity or carbon dioxide.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Clarify samples containing proteins or fat with Carrez clarification.
- Crush or homogenize solid or semi-solid samples. Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Place the volumetric flask in an ice bath for 15 min. to separate the fat, make up to the mark and discard the fatty layer. Filter the sample (water part) or clarify it with Carrez reaction.
- Adjust hard acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.

- Treat strongly coloured samples with Polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml sample).

Carrez-clarification:

Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium – hexacyanoferrate-(II)-trihydrate, 85 mmol = 3.60 g $\text{K}_4[\text{Fe}(\text{CN})_6] \times 3 \text{ H}_2\text{O}/100 \text{ ml}$), 5 ml Carrez-II-solution (zinc sulfate, 250 mmol = 7.20 g $\text{ZnSO}_4 \times 7 \text{ H}_2\text{O}/100 \text{ ml}$) and 10 ml 0.1 M NaOH. Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

Assay procedure for automated systems

Due to a 2-point kinetic measurement this assay is suited only for use on automated photometric systems. If samples have been pre-diluted, the dilution factor has to be considered in the calculation.

Basic parameter for automated photometric systems

Wavelengths	Main 340 nm / Second 415 nm (bichromatic)
Optical path	1 cm
Temperature	37 °C
Measurement	Against reagent blank

	Blank	Sample or calibrator
Sample or calibrator	-	4 µL
Dist. water	4 µL	-
Reagent 1	240 µL	240 µL
Mix, incubate for 300 seconds, then add:		
Reagent 2	60 µL	60 µL
Mix, incubate for 200 seconds, read absorbance (A1):		
Incubate for 120 seconds, read absorbance (A2).		

$$\Delta A = [(A2 - A1) \text{ sample or calibrator}] - [(A2 - A1) \text{ blank}]$$

Calibration

For the calibration of the automated photometric systems, we recommend to use the Enzytec fluid Acid combination Standard (ID-No 5460, 3 x 3 mL). The standard is ready-to-use.

Quality control

For internal quality control of precision and accuracy, appropriate control samples should be used. The frequency of the quality control and the tolerance limits are defined according to the laboratory requirements. The results must be within the defined tolerance limits, and trouble-shooting procedures must be described for the case where the results are outside the specified range.

Performance characteristics

Measuring range

The assay has been developed to determine Acetic acid concentrations up to 1200 mg/L. When values exceed this range, samples should be diluted with dist. water (do not forget the dilution factor in the calculation).

Specificity

The assay is specific for acetic acid. No interference is observed by citric acid up to 2.5 g/L, ascorbic acid up to 1.0 g/L and tartaric acid up to 3.5 g/L.

Lowest detection limit

3 mg/L.
The lowest detection limit corresponds to the smallest Acetic acid concentration differentiating from zero. It is calculated out of three standard deviations from 20 replicates of a zero sample.

Method comparison

A comparison between Enzytec fluid Acetic acid (y) and a commercially available and manual performed test (x) using 26 samples gave following results: $y = 0.947 x + 7.4 \text{ g/L}$; $r = 0.991$.

Precision

Precision in series	n	Mean [mg/L]	SD [mg/L]	CV [%]
Red wine	20	459	2.71	0.59
White wine	20	155	1.86	1.20
Acetate solution	20	98	1.53	1.56

Precision day to day (5 days)	n	Mean [mg/L]	SD [mg/L]	CV [%]
Acetate solution 204 mg/L	20	208	7.01	3.37
Acetate solution 108 mg/L	20	107	4.12	3.85
Acetate solution 301 mg/L	20	306	8.08	2.64
Red wine	20	569	9.31	1.64
White wine	20	214	5.49	2.56

Recovery rate

Recovery rate of spiked wine samples is given in the table below (wine + acetate solution; 9+1).

Probe n = 5	Mean mg/L	Target value mg/L	Recovery %
9+1; white wine 1 + 500mg/L	56	53	106
9+1; white wine 1 + 1000mg/L	106	100	106
9+1; white wine + 2000mg/L	214	200	107
9+1; red wine 1 + 500mg/L	51	53	96
9+1; red wine 1 + 1000mg/L	96	100	96
9+1; red wine 1 + 2000mg/L	189	200	94
9+1; red wine 2 + 500mg/L	54	53	102
9+1; red wine 2 + 1000mg/L	100	100	100
9+1; red wine 2 + 2000mg/L	199	200	99
9+1; white wine 2 + 500mg/L	54	53	102

Waste Management

Please refer to local legal requirements.

Manufacturer

Thermo Fisher Scientific Oy
Ratastie 2, P.O. Box 100, FI-01621 Vantaa, Finland

Distributed by

R-Biopharm AG
An der neuen Bergstrasse 17
D-69297 Darmstadt, Germany

Date of revision (yyyy-mm-dd)

2010-05-06

Konelab / Arena			
Test Definition:			
Testtype	Photometric		
Full name	Acetic acid		
Online name	#		
Result unit	mg/l		
Number of decimals	0		
Acceptance	Automatic		
Dilution 1 +	#		
Sample type	Other		
Temperature	37 °C		
Test in use	Yes		
	Low	High	Unit
Test limit	3	1200	mg/l
Initial absorbance	#	#	E
Dilution limit	#	#	mg/l
Secondary dilution 1 +	#	#	
Correction factor	1.00		
Correction bias	0.00		
Calibration parameter			
Calibration type	Linear		
Repeat time (d)	7		
Points/calibrator	2		
Acceptance	Manual		
Curve direction	Ascending		
Type of calibrators	Separate		
Calibrator identification	Water / Acid combination standard		
Concentration	0 / 200**		
Bias correction in use	No		
Absorbance error (mA)	#		
Relative error (%)	#		
	Min	Max	
Response limit	#	#	
Distribution			
Blank	Fixed timing	Antigen excess	No
Reagent 1	AceR1		
volume (µl)	160		
dispense with	Extra	Volume (µl)	20
wash reagent	None		
Sample volume (µl)	3		
dispense with	Extra	Volume (µl)	10
diluent	Water		
Incubation (sec)	300		
Reagent 2	AceR2		
volume (µl)	40		
wash reagent	None		
dispense with	Extra	Volume (µl)	10
Incubation (sec)	200		
Measurement BLANK	End-point		
response min./max	# / #		
Incubation (sec)	180		
Measurement	End-point		
λ 1 / λ 2 (nm)	340 / 405		
measurement type	Fixed timing		

Data entry by the user

** Target value from the package insert of the acid combination standard. The standard is around 200 mg/l, but the results are extrapolated up to 1200 mg/l because the test is linear up to that level (under the settings described here).

Applications

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TEST	[ACE]
ASSAY CODE	[2] - [40] - [50]
SAMPLE VOLUME (µl)	[4] - [2]
REAGENT VOLUME R1	[240] - [50] - [NO]
REAGENT VOLUME R2	[60] - [20] - [NO]
WAVELENGTH	[405] [340]
CALIBRATION TYPE	[LIN] [1] [2]
STD.(1) CONC. POS.	[0] - [1]
STD.(2) CONC. POS.	[200**] - [2]
STD.(3) CONC. POS.	0 - 0
STD.(4) CONC. POS.	0 - 0
STD.(5) CONC. POS.	0 - 0
STD.(6) CONC. POS.	0 - 0
SD LIMIT	[0.1]
DUPLICATE LIMIT	[100]
SENSITIVITY LIMIT	0
LIMITE D.O (CINET)	[0] - [INCR.]
PROZONE CHECK	[32000] - [1]
EXPECTED VALUE	[3] - [1200]
PANIC VALUE	[#] - [#]
INSTRUMENT FACTOR	[1.0]

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TEST [ACE]	[#]	TEST NAME [ACE]	UNIT [mg/l]
DATA MODE [ON BOARD]		REPORT NAME [ACETIC ACID]	
CONTROL INT [0]		INSTR. FACT. (Y=aX + b) a [1.0]	b [0.0]
EXPECTED VALUE AGE	CLASS 1 M	EXPECTED VALUE F	CLASS 2
[] [] []	- [] []	- [] []	[] []
[] [] []	- [] []	- [] []	[] []
[22] - [29]	[22] - [29]	[] []	[] []
TECHNICAL LIMIT	CLASS 1	CLASS 2	
[] [] []	- [] []	- [] []	[] []
STD	CO N	POS	S.VOL PRE. DIL. VOL
(1) [0.0] [#]	[4]	[0]	[0] [#]
(2) [200**] [#]	[4]	[0]	[0] [#]
(3) [] []	[8]	[]	[] []
(4) [] []	[8]	[]	[] []
(5) [] []	[8]	[]	[] []
(6) [] []	[8]	[]	[] []

TEST	[ACE]
ASSAY CODE	[2 Point end] [10] []
WAVELENGTH (SUB / MAIN)	[415] / [340]
ASSAY POINTS	[25] - [31] - [0] - [0] DILUTION [301] [99]
	< CLASS 1 > < CLASS 2 >
S.VOL (NORMAL)	[4] [0] [0] [] [] []
S.VOL (DECREASE)	[2] [0] [0] [] [] []
S.VOL (INCREASE)	[8] [0] [0] [] [] []
ABS. LIMIT	[0] [32000] [INCREASE]
PROZONE LIMIT	[0] [32000] [UPPER]
REAGENT R1	[240] [0] [#] [#]
R2	[0] [0] [#] [#]
R3	[60] [0] [#] [#]
R4	[0] [0] [#] [#]
CALIB. TYPE	[] LINEAR [] [2] [2] [0] []
AUTOCALIB.	
TIME OUT BLANK	[0] SD LIMIT [999.9]
SPAN	[0] DUPLICATE LIMIT [10000]
2 POINT	[0] SENSITIVITY LIMIT [3]
FULL	[0] SI ABS. LIMIT [-32000][32000]
CHANGE LOT	[NO] COMPENSATED LIMIT []
BOTTLE	[NO]

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Analysis

Test / Type	ACE	Ser/Pl	
Assay / Time / Point	2 Point End	A	10 A 25 31 0 0
Wave (2 nd /Primary)	415 A 340 A		
S.Vol (Normal)	4	0	0
S.Vol (Decrease)	2	0	0
S.Vol (Increase)	8	0	0
Diluent	00951	99	
Reagent (R1) T1	240	0	040 0
Reagent (R2) T2	0	0	040 0
Reagent (R3) T3	60	0	040 0
Reagent (R4) T4	0	0	040 0
Abs. Limit	32000	Increase	A
Prozone Limit	0	32000	Upper A
Cell Detergent	Detergent 1	A	

Calibration

Calibration type	Linear	A	A
Point	2	Span Point	2
Weight	0		
Autocalibration			
Time Out		Change Over	
Blank		Blank	A
Span		Blank	A
2Point			
Full			
SD Limit	200		
Duplicate limit	100	%	32000 Abs
Sensitivity limit	-99999	999999	
S1 Abs limit	-32000	32000	

Range

Application Code	040	Unit	mg/dl	A
Report Name	Acetic acid			
Data Mode	On Board	A		
Control Interval	1000			
Instrument Factor (Y=aX+b)	a= 1.0	b= 0.0		
Technical Limit	3	1500		
Repeat Limit	20	1200		
Expected Value				
(Male)	Y A			
	Y A			
	#	#		
(Female)	Y A			
	Y A			
(Default)	Male	A	Range3	A

Others

<Standard>	(1)	(2)	(3)	(4)	(5)	(6)
Calib. Code	501	#				
Concentration	0	200**				
Position						
Sample Volume	4	4				
Diluent S.Vol	0	0				
Diluent Volume	0	0				

Data entry by the user

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