AAP

COLORIMETRIC DETERMINATION OF AAP
IN URINE
For in vitro diagnostic use only

12 x 6 ml REF **CY01-72**

PRINCIPLE

Alanine aminopeptidase (AAP) is an early marker of brush border damage in proximal tubular cells in renal and interstitial diseases and other diseases.
AAP catalyzes hydrolysis of alanine-p-nitroanilide to alanine and p-nitroaniline.
Released p-nitroaniline is proportional to enzymatic activity.

REAGENTS

Kit components:	REF CY01-72	Quantity
REAGENT 1 (lyophylized)	**CY01-72R1**	**12 vials**

L-alanine-p-nitroanilide 14.8 mmol/L
Buffer pH 7.1 80 mmol/L
Preservative and stabilizers.

STABILITY: stable at 2-8°C up to the expiration date on the label.

PREPARATION OF WORKING REAGENT:

Reconstitute one vial of Reagent 1 with exactly 6.2 mL of distilled water. Mix carefully until complete dissolution.

STABILITY: 48 hours at 2-8°C away from light.

SAMPLE

Urine.

STABILITY: 10 days at 2-8°C. Do not freeze.
Serum: available

MANUAL PROCEDURE

Wavelength:	spectrophotometer 405 nm (400-420 nm)
Pathlength	1 cm
Reading:	against air or distilled water
Temperature:	37°C
Reaction:	30 minutes (see note 4 for kinetic method)
Linearity:	up to 50 U/L
Sample/reagent:	1/20

Pipette into a test tube or cuvette labelled as follows:

	Sample	Blank Reagent
Working reagent	1000 µl	1000 µl

Let it reach 37°C. Add mixing gently:

Sample	50 µl	----
Saline solution	----	50 µl

Read absorbances of sample (As1) and blank reagent (Abr1). After exactly 30 minutes from the first reading, read absorbances of sample (As2) and blank reagent (Abr2).

Calculate the difference of absorbances for the sample and for the blank reagent as follows:
Δ As=As2-As1
Δ Abr=Abr2-Abr1

CALCULATION

AAP activity in (U/L) = (Δ As - Δ Abr) x 71

For activity over 50 U/L dilute 1:10 with saline solution, repeat the determination and multiply the result by 10.

Activity reported as mU/mg of creatinine =
= (U/L x 100) / mg of creatinine for 100 mL.
To evaluate urinary flow of AAP per minute, determine the activity on a urine sample collected during a determined period of time (e.g. 240 minutes).
The mU of AAP excreted per minute is obtained by multiplying the value of the activity of the sample (expressed in U/L) by the volume (in mL) of the urine collected and by dividing it by the minutes of collecting.

EXPECTED VALUES

	MEAN ± DS
Concentration:	10,8 ± 3,8 U/L
	8,1 ± 2,5 mU/mg of creatinine

Excretion rate:

Male	8,4 ± 2,0 mU/min
Female	7,4 ± 1,9 mU/min

NOTES

1. Centrifuge cloudy samples for 5 minutes at 3000 x g.
2. If determinations cannot be run at 405 nm or with 1 cm optical path, a calibration curve with p-nitroaniline can be used.
   It is acceptable to determine only one blank reagent for each series if the values are reproducible.
   Low molecular weight inhibitors in urine can be removed by dialysis. The test has been optimized in order to decrease their activity.
3. Proteases and pH have a great influence on the stability of urinary enzymes. AAP is more stable with pH between 5 and 8. It is recommended to correct acid or alkaline urine pH.
4. **Kinetic procedure:**
   Run the test at 37°C.
   Pipette into test tubes or cuvettes labelled as follows:

	Sample	Blank Reagent
Working reagent	1000 µl	1000 µl
Sample	50 µl	----
Saline solution	----	50 µl

   Read absorbance after 1 min of incubation. Repeat the reading for at least 3 times with intervals of 1 minute.
   Calculate the mean of readings of absorbance/minute for the sample (ΔAs/min) and for the blank reagent(ΔAbr/min).
   To calculate AAP activity in U/L use the following formula:
   AAP activity (U/L) = (Δ As/min - Δ Abr/min) x 2130.
   If activity is over 170 U/L dilute the sample 1:5 with saline solution, repeat the determination and multiply the result by 5.
5. Every laboratory should determine its own reference values.
6. A proportional variation in reagent volumes does not change results.

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The reagents may contain non-reactive components and various preservatives. Contact with the skin and ingestion should be avoided. Use the normal precautions expected with good laboratory practice.

REFERENCES

1. Roger Gibey. et al. Clin. Chim. Acta 116 (1981) 25-34.
2. R.G. Price. Toxicology. 23 (1982) 99-134.

MANUFACTURER

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalogue number
	temperature limits
	use by
	caution
	consult accompanying documents

Issue 01 - 17/11/2023