DIAGNOSTIKA GMBH

Instructions for use

BI-CAT® ELISA

Enzyme Immunoassay for the
Quantitative Determination of
Adrenaline / Noradrenaline in Plasma and Urine

CE IVD

REF EA613/192

2 x 96

2 – 8 °C

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Table of contents

Symbols

IVD	In Vitro Diagnostic Medical Device	CE	EC Declaration of conformity
CONT	Content		Expiry Date
LOT	Lot Number		Store at
	Manufactured by	Σ	Sufficient for ... determinations
REF	Catalogue Number		Consult Instructions for Use

Hazard Pictograms


Warning


Danger

1 Introduction and Principle of the Test

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitter, respectively. Adrenaline and noradrenaline are formed from dopamine. They act on the cardiac musculature and the metabolism (adrenaline) as well as on the peripheral circulation (noradrenaline) and help the body to cope with acute and chronic stress.

An increased production of catecholamines can be found with tumours of the chromaffine system (pheochromocytoma, neuroblastoma, ganglioneuroma). An increased or decreased concentration of the catecholamines can also be found with hypertension, degenerative cardiac diseases, schizophrenia and manic-depressive psychosis.

The assay kit provides materials for the quantitative measurement of adrenaline and noradrenaline in plasma and urine. Noradrenaline and adrenaline are extracted using a cis-diol-specific affinity gel and acylated to N-acylnoradrenaline and N-acyladrenaline and then converted enzymatically into N-acylnormetanephrine and N-acylmetanephrine.

The competitive BI-CAT® ELISA kit uses the microtiter plate format. Adrenaline and noradrenaline, respectively, are bound to the solid phase of the microtiter plate. Acylated catecholamine from the sample and solid phase bound catecholamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase catecholamine is detected by anti-rabbit IgG / peroxidase. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase catecholamine is inversely proportional to the catecholamine concentration of the sample.

2 Precautions

- For in vitro diagnostic use only. For professional use only.
- Some reagents contain sodium azide as preservative. Avoid skin contact.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label.

3 Storage and Stability

On arrival, store the kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.

Do not use components beyond the expiration date shown on the kit labels.

Do not mix various lots of any kit component within an individual assay.

4 Contents of the Kit

4.1 Reagents for sample preparation

Extraction Plate **EX-PLATE** 2 Plates

48 wells. Coated with boronate affinity gel

Extraction-Buffer **EX-BUFF** 1 vial

6 ml, ready for use, colour coded purple

HCl **HCL** 1 vial

21 ml, ready for use, 0.025 M HCl, colour coded yellow orange

Standards (1 - 7) **CAL 1 - 7** 7 vials

Each 4 ml, ready for use,
concentrations:

Standard	1	2	3	4	5	6	7
Adrenaline (ng/ml)	0	0.5	1.5	5	15	50	150
Adrenaline (nmol/l)	0	2.7	8.2	27.3	81.9	273	819
Noradrenaline (ng/ml)	0	1.5	5	15	50	150	500
Noradrenaline (nmol/l)	0	8.9	29.6	88.9	296	887	2,955

When determing urine samples only: Standard 2 can be omitted
When determing plasma samples only: Standard 7 can be omitted

Controls 1 & 2 **CON 1 & 2** 2 vials

Each 4 ml ready for use, concentrations: see Q.C. certificate

Acylation Reagent **ACYL-REAG** 1 vial

6 ml, ready for use, Contains DMSO and DMF
(please note that solvent reacts with many plastic materials including plastic trays; It does not react with normal pipette tips and with glass devices)

Danger
Warning

Acylation Buffer **ACYL-BUFF** 1 vial

20 ml, ready for use, colour coded purple

Enzyme **ENZYME** 3 vials

Each 2 ml, lyoph., Catechol-O-Methyltransferase

Coenzyme **COENZYME** 1 vial

1 ml, gebrauchsfertig, S-Adenosyl-L-Methionin

Enzyme Buffer **ENZYME-BUFF** 1 vial

2 ml, ready for use

Warning

4.2 Reagents for ELISA

Adrenaline Antiserum **AS-AD** 1 vial
6 ml, ready for use, rabbit, colour coded blue

Noradrenaline Antiserum **AS-NAD** 1 vial
6 ml, ready for use, rabbit, colour coded yellow

MT-Strips **STRIPS-AD** 12 strips
8 wells each, break apart, precoated with:
Derivatized adrenaline (12 strips), colour coded blue

MT-Strips **STRIPS-NAD** 12 strips
8 wells each, break apart, precoated with:
Derivatized noradrenaline (12 strips), colour coded yellow

POD Conjugate **CONJ** 2 vials
Each 12 ml, ready for use,
Anti-rabbit IgG-POD Conjugate / peroxidase  Warning

Wash Buffer **WASH** 2 vials
20 ml, concentrate, Dilute content with dist. water to 1000 ml total volume

Substrate **SUB** 2 vials
Each 12 ml TMB solution, ready for use

Stop Solution **STOP** 2 vials
Each 12 ml, ready for use, contains 0.3 M sulphuric acid

Adhesive foil **FOIL** 10 pieces
Ready for use

Additional materials and equipment required but not provided:

- Pipettes for pipetting 20, 50, 300, 1000 µl
- Repeating dispenser for 20, 50, 100, 150, 200, 250 µl und 1 ml
- Horizontal shaker
- Microplate washing device
- Microplate photometer
- Distilled water

5 Sample Collection and Storage

5.1 Plasma

EDTA plasma samples are required for the assay. Physical and psychical stress usually causes a high increase of the catecholamine concentration. Therefore, it is recommended to let the patient rest for 20 to 30 minutes after the venipuncture and before collecting the blood sample.

Haemolytic and especially lipemic samples should not be used for the assay, because false low values will be obtained with such samples.

The plasma samples can be stored at 2 – 8 °C up to 6 hours. For a longer period (up to 1 week) the samples should be stored at -20 °C.

5.2 Urine

The total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 15 ml of 6 M hydrochloric acid as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For patients with suspected kidney disorders the creatinine concentration should be tested, too. Urine samples can be stored at -20 °C for at least 6 months.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

6.1.1 Wash Buffer

Dilute the content of the bottle WASH with distilled water to a total volume of 1000 ml.

Store the diluted wash buffer at 2 - 8 °C for a maximum period of 4 weeks or at -20 °C until the indicated expiry date.

6.1.2 Enzyme Mix

NOTE: The enzyme mix has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). After use the reagent has to be discarded.

Reconstitute the content of one vial labelled ENZYME with 2 ml distilled water.

Add 0.3 ml COENZYME and 0.3 ml ENZYME-BUFF (total volume: 2.6 ml) and mix thoroughly.

The two additional bottles of ENZYME are allowing a second and a third run of the test. If the whole kit is to be used in one run it is recommended to pool the contents of at least two prepared enzyme mix.

6.2 Preparation of Samples

Allow reagents and samples to reach room temperature.

Determinations in duplicates are recommended.

Each 20 µl of Standards, Control 1 & 2 and urine samples are extracted.

Each 300 µl of plasma samples are extracted.

1. Pipette each 20 µl Standard 1 – 7 CAL 1 - 7, 20 µl Control 1 & 2 CON 1 & 2 and each 20 µl Urine Sample into the respective wells of the extraction plate EX-PLATE. Add 250 µl of distilled water to these wells to correct for volume.
   Pipette each 300 µl Plasma Sample into the respective wells (no volume correction required).
2. Pipette 50 µl Extraction Buffer EX-BUFF into each well.
3. Incubate 60 minutes at room temperature on an orbital shaker (400 - 600 r/min).
4. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
5. Pipette 1 ml Wash Buffer WASH into each well and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
6. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
7. Pipette 150 µl Acylation Buffer ACYL-BUFF into each well.
8. Pipette 50 µl Acylation Reagent ACYL-REAG into each wells and continue with step 9. immediately.
   (please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices)
9. Incubate the plate for 20 minutes at room temperature on an orbital shaker (400 - 600 r/min).
10. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.

11. Pipette 1 ml Wash Buffer [WASH] into each well and incubate for 5 minutes at room temperature on an orbital shaker (slow shaking).
12. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.
13. Repeat the wash steps 11. and 12.
14. Pipette 200 µl HCl (0.025 M) [HCL] into each well.
15. Incubate the plate with adhesive foil [FOIL] for 20 minutes at room temperature on an orbital shaker (400 - 600 r/min).

    Caution: Do <u>not</u> decant the supernatant thereafter.

    Take 50 µl each of the supernatants for the adrenaline assay and 50 µl each for the noradrenaline assay.

7 Test Procedure ELISA

Allow reagents to reach room temperature.

7.1 Adrenaline ELISA

1. Pipette each 20 µl of freshly prepared Enzyme Mix into the required number of wells (colour coded blue) STRIPS-AD.
2. Pipette each 50 µl prepared Standards, Controls and Patient Samples into the respective wells.
3. Incubate the plate with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (400 - 600 r/min).
4. Pipette 50 µl Adrenaline-Antiserum (colour coded blue) AS-AD into each well.
5. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 12 – 20 hours (overnight) at 2-8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer WASH. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette 100 µl POD-Conjugate CONJ into each well.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
9. Washing: Repeat wash step 6.
10. Pipette 100 µl Substrate SUB into each well.
11. Shake for 10 seconds, cover with a box and incubate for 30 ± 5 minutes at room temperature (20 – 25 ºC) without shaking.
12. Pipette 100 µl Stop Solution STOP into each well.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

7.2 Noradrenaline ELISA

1. Pipette each 20 µl of freshly prepared Enzyme Mix into the required number of wells (colour coded yellow) STRIPS-NAD.
2. Pipette each 50 µl prepared Standards, Controls and Patient Samples into the respective wells.
3. Incubate the plate with adhesive foil FOIL for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (400 - 600 r/min).
4. Pipette 50 µl Noradrenaline-Antiserum (colour coded yellow) AS-NAD into each well.
5. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 12 – 20 hours (overnight) at 2-8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer WASH. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette 100 µl POD-Conjugate CONJ into each well.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
9. Washing: Repeat wash step 6.
10. Pipette 100 µl Substrate SUB into each well.
11. Shake for 10 seconds, cover with a box and incubate for 30 ± 5 minutes at room temperature (20 – 25 ºC) without shaking.
12. Pipette 100 µl Stop Solution STOP into each well.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8 Calculation of Results

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.

A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

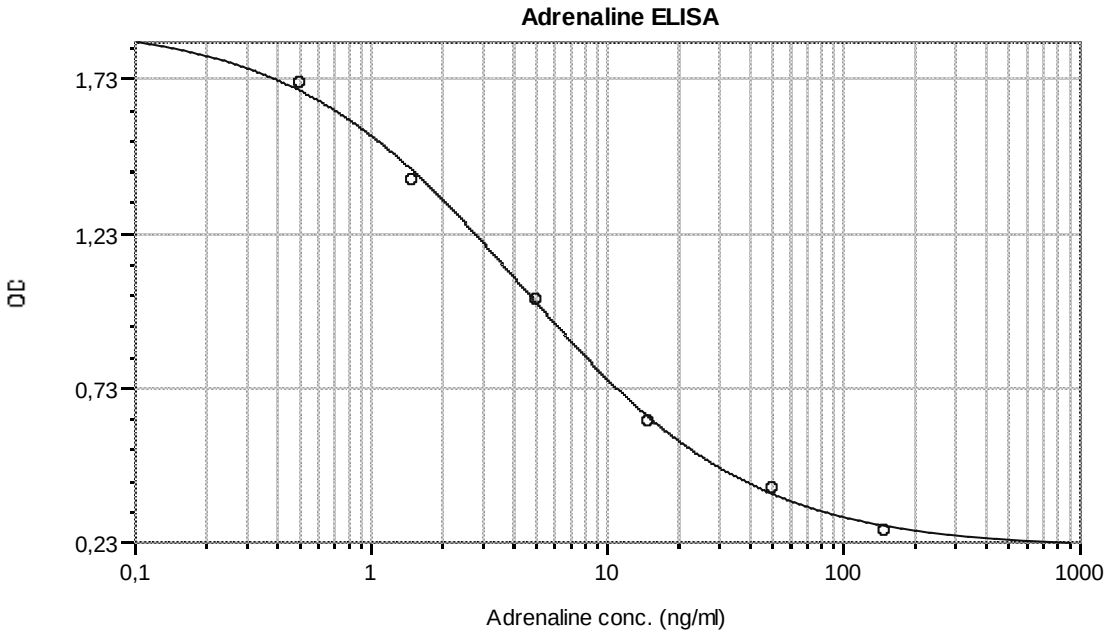
The concentration of the controls and urine samples can be read off the standard curve directly without any further conversion.

The read concentrations of adrenaline and noradrenaline in plasma samples have to be divided by 15 due to the use of 300 µl plasma sample in relation to 20 µl standard.

Typical Examples

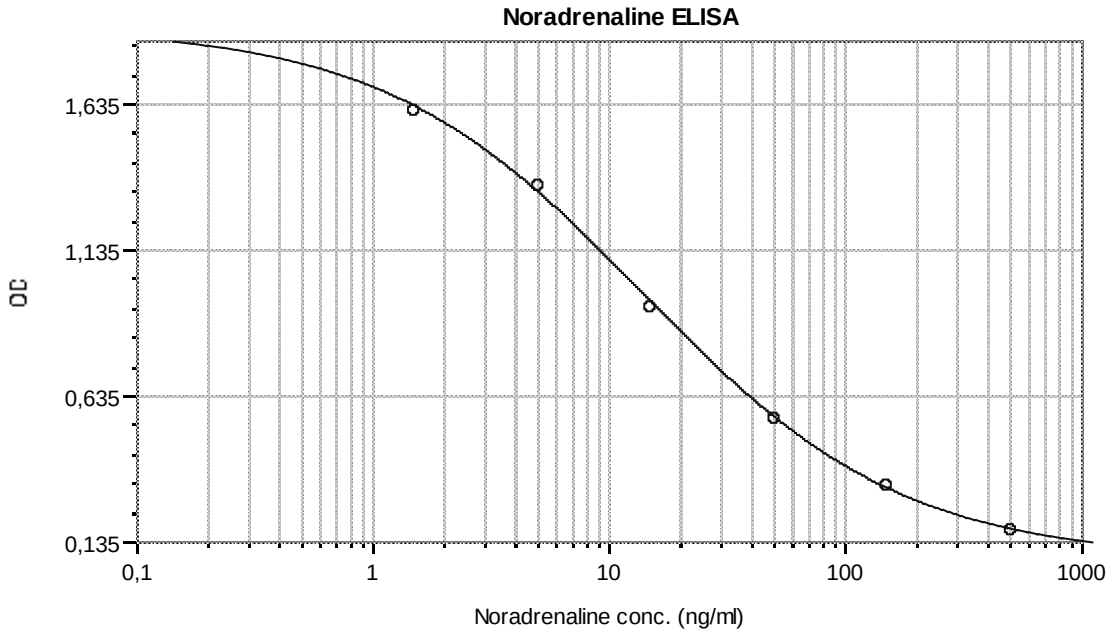
Below are listed typical examples of standard curves with the Adrenaline ELISA and Noradrenaline ELISA:

Adrenaline ELISA



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
○	Std (Standards: Concentration vs MeanValue)	1,908	0,892	4,291	0,216	0,999

Noradrenaline ELISA



	y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
o	Std (Standards: Concentration vs MeanValue)	1,898	0,788	13,744	0,079	1

9 Assay Characteristics

9.1. Reference Ranges

The reference ranges given below should only be taken as a guideline. It is recommended that each laboratory should establish its own normal values.

Matrix	Adrenaline	Noradrenaline
Urine	< 20 µg/day	< 90 µg/day
Plasma	< 100 pg/ml	< 600 pg/ml

9.2. Sensitivity

The lower limit of detection was determined by taking the 2fold standard deviation of the absorbance of the Zero Reference and reading the corresponding value from the standard curve.

Matrix	Adrenaline	Noradenaline
Sensitivity (Urine):	0.08 ng/ml	0.67 ng/ml
Sensitivity (Plasma):	5 pg/ml	45 pg/ml

9.3. Specificity (Cross Reactivity)

Structural related components were tested for possible interference with the antisera against adrenaline and noradrenaline used in the ELISA method.

Components	Cross Reactivity (%) Adrenaline-Ab	Cross Reactivity (%) Noradrenaline-Ab
Adrenaline	100	< 0.01
Noradrenaline	0.053	100
Dopamine	< 0.01	0.37
Metanephrine	< 0.01	< 0.01
Normetanephrine	< 0.001	< 0.01
3-Methoxytyramine	< 0.001	< 0.01
L-Dopa	< 0.001	< 0.01
Tyramine	< 0.001	< 0.01
Tyrosine	< 0.001	< 0.001
Homovanillic acid	< 0.0001	< 0.001
Vanillic mandelic acid	< 0.0001	< 0.001

9.1 Recovery

Increasing amounts of adrenaline and noradrenaline were added to an urine and to a plasma sample. Each spiked sample was assayed. The analytical recovery was estimated at different concentrations by using the theoretically expected and the actually measured values.

Adrenaline

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	2.1 – 30.3	102	100 - 105
EDTA-Plasma	0.02 – 1.39	101	94 – 103

Noradrenaline

Matrix	Range (ng/ml)	Mean (%)	Recovery (%)
Urine	32.4 – 113.2	93	89 - 98
EDTA-Plasma	0.20 – 4.91	104	91 – 109

9.2 Linearity

The linearity of the ELISA method was investigated using different dilutions of an urine and a plasma sample.

Adrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	4.4 – 59.7	1:15 (dist. water)	108	100 - 112
EDTA-Plasma	0.11 – 1.52	1:15 (dist. water)	107	104 - 111

Noradrenaline

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Recovery (%)
Urine	9.9 – 132.3	1:15 (dist. water)	105	98 - 112
EDTA-Plasma	0.33 – 4.87	1:15 (dist. water)	103	100 - 108

9.3 Reproducibility

Adrenaline

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	3.1 – 15.2	7.6 – 7.3 %	2.6 – 16.6	6.7 – 9.6 %
EDTA-Plasma	0.12 – 1.19	9.6 – 9.5 %		

Noradrenaline

Matrix	Range (ng/ml)	Intra-Assay-CV	Range (ng/ml)	Inter-Assay-CV
Urine	21.8 – 76.4	8.7 – 9.2 %	23.1 – 83.9	11.1 – 8.7 %
EDTA-Plasma	0.76 – 4.85	8.4 – 9.7 %		

9.4 Method Comparision

Adrenaline

Matrix	Method	Correlation
Urine	HPLC	Y = 0.94 x HPLC - 0.21; R = 0.987; N = 32

Noradrenaline

Matrix	Method	Correlation
Urine	HPLC	Y = 0.90 x HPLC + 6.3; R = 0.983; N = 32

10 Changes to declare

Version _9: IFU has been re-formatted. The manufacturer and distributor information have been changed. Component names as printed on labels were included in sections 6 and 7 and pipetting schemes to provide greater clarity. No changes have been made to components or execution of protocols.

Pipetting Scheme - Sample Preparation

(Adrenaline, Noradrenaline)

		Standards	Controls	Urine	Plasma
EX-PLATE:					
CAL 1 - 7	µl	20			
CON 1 & 2	µl		20		
Patient Urine	µl			20	
Patient Plasma	µl				300
Dist. Water	µl	250	250	250	
EX-BUFF	µl	50	50	50	50

Incubate 60 minutes at RT (shake: 400 - 600 rpm)
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

ACYL-BUFF	µl	150	150	150	150
ACYL-REAG	µl	50	50	50	50

Immediately shake 20 minutes at RT (shake: 400 - 600 rpm)
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

WASH	ml	1	1	1	1

Incubate 5 minutes at RT (slow shaking)
Decant plate and remove residual liquid

HCL	µl	200	200	200	200

Incubate 20 minutes with FOIL at RT (shake: 400 – 600 rpm)
Caution: Do not decant the supernatant thereafter
For the ELISA take each
50 µl for Adrenaline ELISA
50 µl for Noradrenaline ELISA

Pipetting Scheme - ELISA

		Adrenalin (blue) STRIPS-AD			**Noradrenalin (yellow)** STRIPS-NAD		
		Stand.	Contr.	Samples	Stand.	Contr.	Samples
Enzymemix (fresh)	µl	20	20	20	20	20	20
Acyl. Stand. 1 – 7	µl	50			50		
Acyl. Contr. 1 & 2	µl		50			50	
Acyl. Samples	µl			50			50

Cover with adhesive foil FOIL; shake 30 min at room temperature

AS-AD	µl	50	50	50			
AS-NAD	µl				50	50	50

Cover plates with adhesive foil FOIL
Shake for 10 seconds
Incubate for 12 – 20 hours (overnight) at 2-8°C
4 x washing with 250 µl WASH

CONJ	µl	100	100	100	100	100	100

Incubate for 30 minutes at room temperature on an orbital shaker
4 x washing with 250 µl WASH

SUB	µl	100	100	100	100	100	100

Shake for 10 seconds
Incubate 30 ± 5 minutes at room temperature, covered with a box, without shaking

STOP	µl	100	100	100	100	100	100

Reading of absorbance at 450 nm