



Instructions for Use

Adrenaline ELISA

Enzyme Immunoassay for the
Quantitative Determination of
**Adrenaline in Plasma, Urine,
Cell Culture and other biological Samples**

RUO

For Research Use Only
Not for Use in Diagnostic Procedures

REF

EA604/96



12 x 8



2 – 8 °C












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Table of Contents

1	Introduction and Principle of the Test	4
2	Precautions	5
3	Storage and Stability	5
4	Contents of the Kit	6
5	Sample Collection and Storage	8
6	Preparation of Reagents and Samples.....	11
7	Test Procedure ELISA.....	14
8	Calculation of Results	15
9	Assay Characteristics	17
10	Changes to declare	18
	Pipetting Scheme - Sample Preparation	19
	Pipetting Scheme - ELISA	20

Symbols

 RUO	For Research Use Only		
 CONT	Content		Expiry Date
 LOT	Lot Number		Store at
	Manufactured by		Sufficient for ... determinations
 REF	Catalogue Number		Consult Instructions for Use

The symbols of the components of the kit are described in section 4 Contents of the Kit.

1 Introduction and Principle of the Test

The Adrenaline ELISA kit contains reagents and materials for the quantitative determination of adrenaline in plasma, urine, cell culture samples, tissue homogenates and other samples.

Catecholamine is the name of a group of aromatic amines (noradrenaline, adrenaline, dopamine, and their derivatives) which act as hormones and neurotransmitters, 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.

This assay is for research use only! Not for use in diagnostic procedures!

Adrenaline is extracted using a cis-diol-specific affinity gel and acylated to N-acyladrenaline and then converted enzymatically into N-acylmetanephrine.

The competitive Adrenaline ELISA kit uses the microtiter plate format. Adrenaline is bound to the solid phase of the microtiter plate. Acylated adrenaline from the sample and solid phase bound adrenaline 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 adrenaline is detected by peroxidase-conjugated anti-rabbit IgG. The substrate TMB / peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase adrenaline is inversely proportional to the adrenaline concentration of the sample.

2 Precautions

- For research use only. For professional use only!
- Before carrying out the test, the instructions for use, as included in the kit, should be read completely and the content understood.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy; however these materials should be handled as potentially infectious.
- Individual components of different lots and test kits should not be interchanged. The expiry dates and storage conditions stated on the packaging and the labels of the individual components must be observed.
- When handling the reagents, controls and samples, the current laboratory safety guidelines and good laboratory practice should be observed.
- Wear protective clothing, disposable gloves, and eye protection while performing the test.
- Some components of this kit are containing hazardous reagents. These components are marked with the adequate hazard label. Further information is in section 4 and in the corresponding MSDS.
- Avoid any actions that could result in ingestion, inhalation or injection of the reagents. Never pipette by mouth.
- Avoid contact with individual reagents, these can cause irritation and chemical burns.
- Dispose of waste according to state and local environmental protection regulations.

3 Storage and Stability

The kit is shipped at ambient temperature and is subsequently stable until the stated expiry date when stored between 2 – 8 °C. Once opened, the kit is stable until the expiry date.

The shelf life of the ready-to-use reagents is indicated on the respective bottle label. The shelf life and storage conditions of the prepared reagents is stated under 6.1.

Bring all reagents to room temperature before use and refrigerate immediately after use.

4 Contents of the Kit

4.1 Reagents for Sample Preparation:

Extraction Plate EX-PLATE 2 plates
48 wells each, coated with boronate affinity gel

Extraction Buffer EX-BUFF 2 vials
6 ml each, ready for use



HCl HCL 1 vial
13 ml, ready for use, 0.025 M HCl

Standards 1 – 6 CAL 1 - CAL 6 6 vials
4 ml each, ready for use, Concentrations:

CAL		1	2	3	4	5	6
Adrenaline	(ng/ml)	0	0.15	0.5	1.5	5	15
	(nmol/l)	0	0.82	2.7	8.2	27.3	81.9

Control 1 & 2 CON 1 & CON 2 2 vials
4 ml each, ready for use
Concentration: see QC Certificate





Acylation Reagent ACYL-REAG 1 vial
6 ml, ready for use, contains DMSO and DMF;
(Please note that DMSO/DMF reacts with many plastic materials including plastic trays. It does not react with normal pipette tips and with glass devices)


Warning
 
Danger

Acylation Buffer ACYL-BUFF 1 vial
20 ml, ready for use

Enzyme ENZYME 3 vials
2 ml each, lyophilized, Catechol-O-Methyltransferase

Coenzyme COENZYME 1 vial
1.75 ml, ready for use, S-Adenosyl-L-Methionine

Enzyme Buffer 2 ml, ready for use	ENZYME-BUFF  Warning	1 vial
Sample Stabilizer 20 ml, ready for use	STABILIZER  Warning	1 vial
4.2 Reagents for ELISA:		
Adrenaline-Antiserum 4 ml, ready for use, rabbit, color codes blue	AS-AD  Warning	1 vial
MT-Strips 8 wells each, break apart, precoated with: adrenaline, color coded blue	STRIPS-AD	12 strips
Enzyme Conjugate 12 ml, ready for use, Anti-rabbit IgG-Peroxidase	CONJ  Warning	1 vial
Wash Buffer 20 ml each, concentrated Dilute contents with dist. water to 1000 ml total volume	WASH	2 vials
Substrate 12 ml, TMB solution, ready for use	SUB	1 vial
Stop Solution 12 ml, ready for use, contains 0.3 M sulphuric acid	STOP	1 vial
Adhesive foil Ready for use	FOIL	10 pieces

Additional materials and equipment required but not provided:

- Pipettes for pipetting 20, 90, 500, 1000 μ l
- Repeating dispenser for 30, 50, 100, 150, 250 μ l, 1000 μ l
- Horizontal shaker
- Microplate washing device or multichannel pipette
- Microplate photometer (450 nm)
- Distilled water
- Paper towels, pipette tips, timer

5 Sample Collection and Storage

5.1 Plasma

EDTA plasma samples are required for the assay.

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

Immediately after collection, the plasma samples should be centrifuged (preferably at 2 - 8 °C) and frozen. The samples are stable up to 1 week at -20 °C. Storage at -80 °C should be preferred, if possible.

To improve the stability, each sample should be enriched with the Sample Stabilizer [STABILIZER] before freezing (add 20% of the sample volume), e.g.:

Sample volume	+ Stabilizer volume	= Total volume
20 μ l	4 μ l	24 μ l
50 μ l	10 μ l	60 μ l
100 μ l	20 μ l	120 μ l
200 μ l	40 μ l	240 μ l
300 μ l	60 μ l	360 μ l
500 μ l	100 μ l	600 μ l

Note: Multiply the determined Adrenaline concentration of the sample by the factor 1.2 (see section 8).

We recommend using EGTA/Gluthation (GSH) blood collection tubes. The addition of the Sample Stabilizer is then not necessary.

Before use, mix and centrifuge (10 minutes, 2,000 xg) stabilized plasma samples

In general, mix and centrifuge (10 minutes, 2,000 xg) plasma samples after thawing.

5.2 Urine Samples

Spontaneous urine and collected urine can be used.

The urine samples must be acidified to stabilize them.

Spontaneous urine:

Acidify urine with hydrochloric acid, e.g. 15 ml urine + 100 µl 6 mol/l HCL.

Collected urine:

The total volume of urine excreted during a 24-hour period should be collected in a single bottle containing 10 – 15 ml of 6 N hydrochloric acid as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. With suspected kidney disorders, the creatinine concentration should be determined in addition.

Urine samples can be stored at -20 °C for at least 6 months.

Before use, mix and centrifuge (e.g. 3 minutes, 3,000 xg) urine.

Further, dilute urine samples 1:5 with dist. water.

5.3 Cell culture samples and various biological samples

The storage and stability of such samples depends on the type of sample and how they are collected. Therefore, only a general procedure for collection and storage can be outlined:

It is recommended to freeze the samples immediately after collection. The samples should be stable at -20 °C for up to 1 week. Storage at -80 °C should be preferred, if possible.

To improve the stability, each sample should be enriched with the Sample Stabilizer **STABILIZER** before freezing (10% of the sample volume), e.g.:

Sample volume	+ Stabilizer volume	= Total volume
20 µl	2 µl	22 µl
50 µl	5 µl	55 µl
100 µl	10 µl	110 µl
200 µl	20 µl	220 µl
300 µl	30 µl	330 µl
500 µl	50 µl	550 µl

Note: Multiply the determined Adrenaline concentration of the sample by the factor 1.1 (see section 8).

Acidified samples, which have a pH value of 5 or less must not be enriched with the Sample Stabilizer and must be frozen immediately after collection.

5.4 Tissue samples

Tissue samples can be homogenized in Sample Stabilizer **STABILIZER** diluted 1:20 (e.g. 19 ml dist. water + 1 ml Sample Stabilizer).

For all sample types, the following basic principles should be followed:

- Avoid excess of acid. This might exceed the buffer capacity of the extraction buffer **EX-BUFF**. After adding the extraction buffer (see 6.2) a pH value of 7 or above is mandatory, otherwise binding of adrenaline to the boronate affinity gel will not take place. If the pH value is below 7 it is necessary to repeatedly add 50 µl of Extraction Buffer **EX-BUFF** until the pH value is at or above 7. The pH can be determined by pipetting 0.5 µl on pH indicator paper.
Acidified samples, which have a pH value of 5 or less must not be enriched with the Sample Stabilizer and have to be frozen immediately after collection.
- Avoid substances in the samples with a cis-diol-structure (boric acid, sorbitol, mannitol, etc.). These substances reduce the recovery of extraction and lead to false low values.

6 Preparation of Reagents and Samples

6.1 Preparation of Reagents

Allow reagents to reach room temperature.

6.1.1 Wash Buffer

Dilute the content (20 ml, 50x) of the bottle **WASH** with distilled water to a total volume of 1000 ml, **mix briefly**.

Store the diluted wash buffer at 2 – 8 °C for a maximum period of 4 weeks. Should the kit be used in several runs, then prepare only the required amount of wash buffer for each run.

6.1.2 Enzyme Mix

NOTE: The enzyme mix must be prepared freshly prior to the assay (no longer than **20 – 30** minutes in advance). After use, discard the reagent.

Reconstitute the content of one vial **ENZYME** with 2 ml distilled water. Add **0.5 ml** **COENZYME** and **0.5 ml** **ENZYME-BUFF** (total volume: **3.0** ml) and mix thoroughly.

The two additional bottles of **ENZYME** allow a second and a third run of the test. If the whole kit is to be used in one run, **two bottles of enzyme mix need to be prepared and pooled** (in e.g. a 10 ml test tube).

All other reagents are ready for use.

6.2 Preparation of Samples

Allow reagents and samples to reach room temperature.

Determinations in duplicates are recommended.

Each 20 µl of Standards, Controls and Urine Samples (diluted 1:5) are extracted. Each 300 µl of plasma samples are extracted. Of other sample types 1 µl up to 300 µl can be extracted.

1. Pipette each 20 µl Standard 1 – 6 [CAL 1] - [CAL 6] , 20 µl Control 1 & 2 [CON 1] & [CON 2] and 20 µl Urine Sample (diluted 1:5 with dist. water) into the respective wells of the extraction plate [EX-PLATE]. Add 250 µl of distilled water to these wells to correct for volume.

Pipette 300 µl Plasma Sample into the respective wells (no volume correction required).

Pipette 1 µl up to 300 µl of other sample types into respective wells and correct volume of each well to 300 µl with distilled water, e.g. 100 µl sample + 200 µl dist. water).

2. Add 100 µl Extraction Buffer [EX-BUFF] into each well.
3. Incubate 60 minutes at room temperature on an orbital shaker at medium speed.
4. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.
5. Pipette 1 ml Wash Buffer (see 6.1.1) into each well and incubate for 5 minutes at room temperature on an orbital shaker at low speed.
6. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.
7. Pipette 150 µl Acylation Buffer [ACYL-BUFF] into each well.
8. Add 50 µl Acylation Reagent [ACYL-REAG] into each well and continue with next step, 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 at medium speed.
10. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.

11. Pipette 1 ml Wash Buffer (see 6.1.1) into each well and incubate for 5 minutes at room temperature on an orbital shaker at low speed.
12. Decant the plate and remove residual liquid by firmly tapping the inverted plate on a paper towel.
13. Repeat the wash steps 11. and 12 twice.
14. Pipette 100 μ l HCl [HCL] into each well to elute the Adrenaline.
15. Incubate the plate sealed with adhesive foil [FOIL] for 20 minutes at room temperature on an orbital shaker at medium speed.
Caution: Do not decant the supernatant.
16. Add 50 μ l of freshly prepared Enzyme Mix (s. 6.1.2) into each well. A color change to red occurs and indicates which wells have already been pipetted.
17. Incubate the plate sealed with adhesive foil [FOIL] for 30 minutes at room temperature on an orbital shaker at medium speed.
Caution: Do not decant the supernatant.

Take 90 μ l of each supernatant for the Adrenaline ELISA.

7 Test Procedure ELISA

1. Transfer each 90 μ l prepared Standards, Controls and Samples into the STRIPS – AD wells (color coded blue). Thereby, slightly tilt EX-PLATE to facilitate pipetting the supernatants.
2. Add 30 μ l Adrenaline-Antiserum AS-AD (color coded blue) into each well.
3. Cover the plate with adhesive foil FOIL, shake for 10 seconds and incubate for 15 - 20 hours (overnight) at 2 – 8 °C.
4. Discard or aspirate the contents of the wells and wash thoroughly with each 250 μ l Wash Buffer (see 6.1.1). Remove residual liquid by firmly tapping the inverted plate on clean absorbent paper towel. Repeat the washing procedure 3 times.
5. Pipette 100 μ l Enzyme Conjugate CONJ into each well.
6. Incubate for 30 minutes at room temperature on an orbital shaker at medium speed.
7. Washing: Repeat wash step 4.
8. Pipette 100 μ l Substrate SUB into each well.
9. Shake plate for 10 seconds on orbital shaker, then place on table, cover with a box and incubate for 30 \pm 5 minutes at room temperature (20 - 25 °C) without shaking.
10. Add 100 μ l Stop Solution STOP into each well. Shake plate for 10 seconds on orbital shaker.
11. 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

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).

Correcting read concentrations:

Controls: The concentrations can be read off the standard curve directly without any further conversion.

Urine Samples: The read concentrations must be multiplied by 5, due to the 1:5 dilution of the samples.

Plasma Samples: The read concentrations must be divided by 15, due to the use of 300 μ l plasma sample in relation to 20 μ l per standard. Further, when 20% Sample Stabilizer (see 5.1) was added to the samples, the read concentration must be multiplied by 1.2.

Other Sample Types: As sample volumes of 1 μ l up to 300 μ l can be used, the read concentrations must be divided by a volume factor calculated as following

$$\text{Volume factor} = \frac{\text{Sample volume used for extraction } [\mu\text{l}]}{20 \mu\text{l (Volume of Standards)}}$$

Example:

200 μ l Sample were used for extraction and the read concentration is 0.6 ng/ml.

Volume factor is $200 \mu\text{l} / 20 \mu\text{l} = 10$

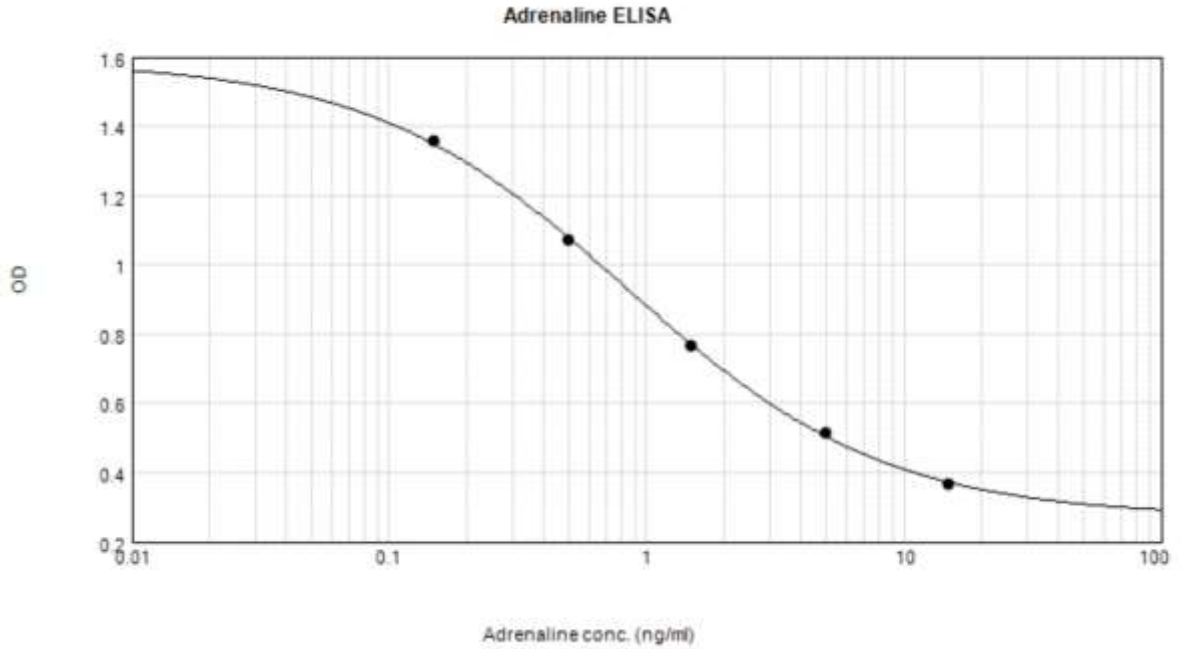
Concentration of sample is $0.6 \text{ ng/ml} / 10 = 0.060 \text{ ng/ml} = 60 \text{ pg/ml}$

Further, when 10% Sample Stabilizer (see 5.3) was added to the samples, the concentration must be multiplied by 1.1.

Adrenaline: $1 \text{ ng/ml} = 5.46 \text{ nmol/l}$

Quality Control: Test results are valid only if the kit controls are within the ranges specified on the QC Certificate. Otherwise, the test should be repeated.

Typical Example (do not use for calculation of results):



9 Assay Characteristics

9.1 Assay Data

9.1.1 Sensitivity

Matrix	LLOD Adrenaline	Calculation
Urine:	0.27 ng/ml	$OD_{Cal1} - 2xSD$
EDTA-Plasma:	3.6 pg/ml	$OD_{Cal1} - 2xSD$

9.1.2 Specificity (Cross Reactivity)

Components	Cross Reactivity (%)
Adrenaline	100
Noradrenaline	0.085
Dopamine	0.00041
Metanephrine	0.0013
Normetanephrine	< 0.0001
3-Methoxytyramine	< 0.0001
L-Dopa	< 0.0001
Tyramine	< 0.0001
Tyrosine	< 0.00005
Homovanillic acid	< 0.00001
Vanillic mandelic acid	< 0.00001

9.1.3 Recovery after spiking

Matrix	Range (ng/ml)	Mean (%)	Range (%)
Urine	2.1 – 25.8	105	102 – 110
EDTA-Plasma	0.008 – 0.237	92	87 – 98

9.1.4 Linearity

Matrix	Range (ng/ml)	Max. dilution	Mean (%)	Range (%)
Urine	2.6 – 23.1	1:10 (dist. water)	103	93 - 112
EDTA-Plasma	0.04 – 0.245	1:7 (dist. water)	107	103 - 113

9.1.5 Reproducibility

Matrix	Range (ng/ml)	Intra-Assay-CV
Urine	3.9 – 14.3	8.5 – 6.4 %
EDTA-Plasma	0.044 – 0.148	11.3 – 7.1 %

9.1.6 Method Comparison

Matrix	Method	Correlation
Urine	HPLC	$Y = 1.12 \times \text{HPLC} - 3.7$; $R = 0.982$; $N = 32$

9.2 Calibration

The calibration of the Adrenaline ELISA is carried out by weighing the pure substance. The correctness of the method was determined by comparison of method (see 9.1.6).

9.3 Limitations of Method

Samples measured above the highest standard must be diluted with the appropriate medium as stated in 9.1.4 and re-assayed. The values of diluted samples must be multiplied by the appropriate dilution factor.

9.4 Interferences

Hemolytic, lipemic and icteric specimens should not be used in the Adrenaline ELISA. Do not use non-acidified urine collection.

10 Changes to declare

Version _11 (valid as of lot A129): Changes are highlighted in grey

Version _10 (valid as of lot A122): Hazard symbol was removed from POD Conjugate. Further changes are highlighted in grey.

Pipetting Scheme - Sample Preparation

		Standards	Controls	Urine	Plasma	Other
EX-PLATE:						
CAL 1 – 6	µl	20				
CON 1 & 2	µl		20			
Urine (1:5 dil.)	µl			20		
Plasma	µl				300	
Other Types	µl					1 to 300
Dist. Water	µl	250	250	250		299 to 0
EX-BUFF	µl	100	100	100	100	100

Shake 60 minutes at RT

Decant plate and remove residual liquid

Wash Buffer	ml	1	1	1	1	1
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Incubate 5 minutes at RT (slow shaking)

Decant plate and remove residual liquid

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

Immediately, shake 20 minutes at RT

Decant plate and remove residual liquid

Wash Buffer	ml	1	1	1	1	1
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Incubate 5 minutes at RT (slow shaking)

Decant plate and remove residual liquid

Repeat washing steps 2x

Decant plate and remove residual liquid

HCL	µl	100	100	100	100	100
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Cover with **FOIL**; shake 20 minutes at RT

Enzyme Mix (fresh)	µl	50	50	50	50	50
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Cover with **FOIL**; shake 30 minutes at RT**Caution: Do not decant the supernatant thereafter**

Use 90 µl for the Adrenaline ELISA

Pipetting Scheme - ELISA

		Prepared Standards	Prepared Controls	Prepared Samples
STRIPS-AD (blue):				
Transfer from EX-PLATE into STRIPS-AD	μl	90	90	90
AS-AD (blue)	μl	30	30	30

Cover the plate with **FOIL**

Shake for 10 seconds

Incubate for 15 – 20 hours (overnight) at 2 – 8 °C

Decant plate and remove residual liquid

Wash Buffer	μl	250	250	250
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Decant plate and remove residual liquid

Repeat washing steps 3x

Decant plate and remove residual liquid

CONJ	μl	100	100	100
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Shake 30 minutes at RT

Decant plate and remove residual liquid

Wash Buffer	μl	250	250	250
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Decant plate and remove residual liquid

Repeat washing steps 3x

Decant plate and remove residual liquid

SUB	μl	100	100	100
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Shake for 10 seconds

Incubate 30 ± 5 minutes at RT, covered with a box, without shaking

STOP	μl	100	100	100
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Shake for 10 seconds

Read absorbance at 450 nm (ref. 570 – 650 nm)