

ORGENTEC Diagnostika GmbH

Carl-Zeiss-Straße 49-51
55129 Mainz - Germany

Phone: +49 (0) 61 31 / 92 58-0
Fax: +49 (0) 61 31 / 92 58-58
Internet: www.orgentec.com



Instruction For Use
2014-01

USA: PMS No. K962926

ORG 515S Anti-Cardiolipin Screen

NAME AND INTENDED USE

Anti-Cardiolipin Screen is an ELISA test system for the quantitative measurement of IgG, IgM and IgA class autoantibodies against cardiolipin in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device	MICROPLATE	Microplate
Manufacturer		CONTROL A	Control
REF	Catalogue number	CONTROL B	Control
96	Sufficient for 96 determinations	CONTROL C	Control
LOT	Batch code	CONTROL D	Control
Use by			
Temperature limitation			
Consult instructions for use		DILUENT	Sample Buffer P
Keep away from sunlight		CONJUGATE GAM	Enzyme Conjugate
Do not reuse		TMB	TMB Substrate
Date of manufacture		WASH	Stop solution
CE	conform to European directive 98/79/EC	STOP	Wash Buffer
		RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SUMMARY AND EXPLANATION OF THE TEST

Anti phospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage, and intrauterine foetal death. Clinical symptoms are accompanied by the occurrence of specific autoantibodies that are detectable in the blood of patients with APS. These antibodies bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I.

The clinical symptoms of APS alone are not sufficiently specific to make a definitive diagnosis. Laboratory tests thus play an important role in the diagnosis of the disease. The Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis defined clinical criteria and diagnostically relevant laboratory parameters in the Sapporo Criteria for the classification of anti phospholipid syndrome, published in 1999. These were revised and updated in 2006 and 2012.

They include the following laboratory parameters:

1. Detection of lupus anticoagulant (LA) in the plasma twice in the space of twelve weeks, according to the guidelines of the International Society on Thrombosis and Hemostasis.
2. Elevated anti-cardiolipin titre (IgG and/or IgM) in the blood. The values must be determined on two occasions at least twelve weeks apart using standardized ELISA tests for beta-2-glycoprotein I dependent cardiolipin antibodies.
3. Elevated beta-2-glycoprotein I antibody titre (IgG and/or IgM). The values must be determined on two occasions at least twelve weeks apart. Detection is performed by means of a standardized ELISA test.

The diagnosis of APS is considered as confirmed when at least one clinical and one of the laboratory criteria are fulfilled.

In primary APS autoantibodies against phospholipids appear independently, while in secondary APS phospholipid antibodies are detected in conjunction with other autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome. Phospholipid antibodies are detectable in only 1-5 % of healthy individuals, but they are found in 16-35 % of lupus patients.

The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombosis and thrombocytopenia. In gynaecology they are supposed to cause intrauterine death or recurrent abortion. Furthermore, anti-cardiolipin antibodies have been detected in neurological disorders like cerebrovascular insufficiency, cerebral ischemia, epilepsy or chorea.

Anti-cardiolipin autoantibodies occur in the immunoglobulin classes IgG, IgM or IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune diseases, whereas IgG antibodies are present in progressive stages of manifested autoimmune disorders. The determination of IgA antibodies seems to have a greater importance in the African-Caribbean population.

Quantitative measurement of anti-cardiolipin antibodies, especially IgG, shows high specificity in therapy-monitoring of secondary APS related to SLE.

Clinical indications for determination of anti-cardiolipin antibodies are: SLE, thrombosis, thrombocytopenia, cerebral ischemia, chorea, epilepsy, recurrent abortion, intrauterine death.

The discovery that anti-phospholipid antibodies recognize plasma proteins that are associated with phospholipids rather than binding to the phospholipids themselves has been a major advance in APS research. Several reports indicate that beta-2-glycoprotein I antibodies are clinically relevant. Recent studies suggest the presence of a dominant epitope on the first domain of beta-2-glycoprotein I. In contrast to antibodies recognizing other domains of beta-2-glycoprotein I, anti-domain I antibodies are found to be highly associated with clinical symptoms.

Anti-cardiolipin and anti-beta-2-glycoprotein I antibodies are independent risk factors for the occurrence of vascular thrombosis and pregnancy loss. However, patients testing positive for multiple antibody specificities generally have a more severe disease and higher recurrence rates despite treatment.

Besides the standardized laboratory assays for detection of anti-cardiolipin antibodies, antibodies directed to beta-2-glycoprotein I and LA, defined in the classification criteria, several other autoantibodies have shown to be relevant to APS. Among them are antibodies against negatively-charged phospholipids, like phosphatidyl serine, phosphatidyl inositol and phosphatidic acid (PA). These antigens can improve the clinical sensitivity in patient samples with suspected APS but they will not replace the determination of autoantibodies against cardiolipin or beta-2-glycoprotein I.

Autoantibodies that bind to proteins of the coagulation cascade or complexes of these proteins with phospholipids have also been proposed to be relevant for APS. As an example, a test for anti-prothrombin antibodies in conjunction with other parameters may be a good risk marker for thrombosis. Antibodies to Annexin V may also be detectable within the clinical framework of APS with otherwise negative phospholipid antibody results.

CONTENTS OF THE KIT

ORG 515S	▽ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: CLP
CONTROL A	1x 1.5 ml	Control A 3.3 U/ml, containing serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL B	1x 1.5 ml	Control B 10 U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL C	1x 1.5 ml	Control C 30 U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL D	1x 1.5 ml	Control D 90 U/ml, containing Cardioliipin antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE GAM	15 ml	Enzyme Conjugate; containing anti-human IgG, IgA and IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%; light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
STOP	15 ml	Stop solution; contains acid. Ready to use.
i	1	Instruction for Use: ELISA Mini-DVD
i	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and dessicated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production.
Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
We recommend consumption on the same day.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.

- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:
Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex.
Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells.

Incubate for **30 minutes** at room temperature (20-28 °C).

Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.

- Dispense **100 µl** of enzyme conjugate into each well.
Incubate for **15 minutes** at room temperature.
Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
- Dispense **100 µl** of TMB substrate solution into each well.
Incubate for **15 minutes** at room temperature
- Add 100 µl** of stop solution to each well of the modules
Incubate for **5 minutes** at room temperature.
Read the optical density at 450 nm (reference 600-690nm) and calculate the results.
The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A											
B	B											
C	C											
D	D											
E	P1											
F	P2											
G	P3											
H	P4											

P1, ... patient sample A, B, C, D controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.
If these quality control criteria are not met the assay run is invalid and should be repeated.

CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville and the specific reference material IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range

The calculation range of this ELISA assay is 0 - 90 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/ml

Interpretation of results

Negative: < 10 U/ml
Positive: ≥ 10 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	91.0	91.0	100
.	1:200	47.7	45.5	105
.	1:400	24.0	22.8	105
.	1:800	11.5	11.4	101
.	1:1600	6.0	5.7	105
2	1:100	69.1	69.1	100
.	1:200	33.5	34.6	97
.	1:400	15.9	17.3	92
.	1:800	7.8	8.6	91
.	1:1600	4.1	4.3	95

Limit of detection

Functional sensitivity was determined to be: 1 U/ml

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	5.8	12.4
2	15.8	8.1
3	43.7	4.7
4	75.9	2.5

Inter-Assay		
Sample	Mean U/ml	CV %
1	4.5	13.3
2	14.8	5.2
3	42.1	4.1
4	70.2	6.6

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
primary APS	8	8	100.0
secondary APS	65	61	93.8
normal human serum	150	8	5.3

		Clinical Diagnosis		
		Pos	Neg	
ORG 515S	Pos	69	8	73
	Neg	4	142	
				223

Sensitivity: 94.5 %
Specificity: 94.7 %
Overall agreement: 94.6 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Banzato A, Pozzi N, Frasson R, De F, V, Ruffatti A, Bison E et al. Antibodies to Domain I of beta(2)Glycoprotein I are in close relation to patients risk categories in Antiphospholipid Syndrome (APS). *Thromb Res* 2011; 128(6):583-6.
2. Bertolaccini ML, Amengual O, Atsumi T, Binder WL, de LB, Forastiero R et al. 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010. *Lupus* 2011; 20(2):191-205.
3. de Laat B, de Groot PG. Autoantibodies directed against domain I of beta2-glycoprotein I. *Curr Rheumatol Rep* 2011; 13(1):70-6.
4. de Laat B, Mertens K, de Groot PG. Mechanisms of disease: antiphospholipid antibodies-from clinical association to pathologic mechanism. *Nat Clin Pract Rheumatol* 2008; 4(4):192-9.
5. de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE et al. The association between circulating antibodies against domain I of beta2-glycoprotein I and thrombosis: an international multicenter study. *J Thromb Haemost* 2009; 7(11):1767-73.
6. Espinosa G, Cervera R. Antiphospholipid syndrome. *Arthritis Res Ther* 2008; 10(6):230.
7. Favaloro EJ, Wong RC. Laboratory testing for the antiphospholipid syndrome: making sense of antiphospholipid antibody assays. *Clin Chem Lab Med* 2011; 49(3):447-61.
8. Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. *Arthritis Rheum* 2007; 27(1):35-46.
9. Giannakopoulos B, Passam F, Ioannou Y, Krihis SA. How we diagnose the antiphospholipid syndrome. *Blood* 2009; 113(5):985-94.
10. Greaves M, Cohen H, Machin SJ, Mackie I. Guidelines on the investigation and management of the antiphospholipid syndrome. *Br J Haematol* 2000; 109(4):704-15.
11. Hughes GR. Hughes syndrome: antiphospholipid syndrome. *J R Coll Physicians Lond* 1998; 32(3):260-4.
12. Hughes GR. Hughes Syndrome (the antiphospholipid syndrome): ten clinical lessons. *Autoimmun Rev* 2008; 7(3):262-6.
13. Hughes GR. Antiphospholipid syndrome, migraine and stroke. *Lupus* 2010; 19(5):555-6.
14. Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. *J Rheumatol* 1986; 13(3):486-9.
15. Koike T, Bohgaki M, Amengual O, Atsumi T. Antiphospholipid antibodies: lessons from the bench. *J Autoimmun* 2007; 28(2-3):129-33.
16. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. *Arthritis Rheum* 2012; 64(1):1-10.
17. Mackworth-Young C. Primary antiphospholipid syndrome: a distinct entity? *Autoimmun Rev* 2006; 5(1):70-5.
18. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006; 4(2):295-306.
19. Molina JF, Gutierrez-Urena S, Molina J, Uribe O, Richards S, De CC et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J Rheumatol* 1997; 24(2):291-6.
20. Oku K, Atsumi T, Amengual O, Koike T. Antiprothrombin antibody testing: detection and clinical utility. *Semin Thromb Hemost* 2008; 34(4):335-9.
21. Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with anticardiolipin and anti-beta2-Glycoprotein I activity. *Thromb Res* 2007; 120(1):127-33.
22. Pierangeli SS, de Groot PG, Dlott J, Favaloro E, Harris EN, Lakos G et al. 'Criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, Texas, April 2010. *Lupus* 2011; 20(2):182-90.
23. Pierangeli SS, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC et al. Standards and reference materials

for the anticardiolipin and anti-beta-2-glycoprotein I assays: a report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. *Clin Chim Acta* 2012; 413(1-2):358-60.

24. Sinico RA, Bollini B, Sabadini E, Di Toma L, Radice A. The use of laboratory tests in diagnosis and monitoring of systemic lupus erythematosus. *J Nephrol JID - 9012268* 2002; 15 Suppl 6:S20-S27.
25. Tincani A, Andreoli L, Casu C, Cattaneo R, Meroni P. Antiphospholipid antibody profile: implications for the evaluation and management of patients. *Lupus* 2010; 19(4):432-5.
26. Tincani A, Morozzi G, Afeltra A, Alessandri C, Allegri F, Bistoni O et al. Antiprothrombin antibodies: a comparative analysis of homemade and commercial methods. A collaborative study by the Forum Interdisciplinare per la Ricerca nelle Malattie Autoimmuni (FIRMA). *Clin Exp Rheumatol* 2007; 25(2):268-74.
27. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum* 1999; 42(7):1309-11.
28. Wong RC, Favaloro EJ, Adelstein S, Baumgart K, Bird R, Brighton TA et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. *Pathology* 2008; 40(1):58-63.
29. Wong RC, Gillis D, Adelstein S, Baumgart K, Favaloro EJ, Hendle MJ et al. Consensus guidelines on anticardiolipin antibody testing and reporting. *Pathology* 2004; 36(1):63-8.



Distributed By:

IBL-America, Inc.

8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA

info@ibl-america.com

(888) 523 1246

- 1 **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
→ **30 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- 2 **100 µl** Enzymkonjugatlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
→ Inhalt der Platte verwerfen und
3 mal mit **300 µl** Waschpuffer waschen
- 3 **100 µl** Substratlösung pipettieren
→ **15 Minuten** bei Raumtemperatur inkubieren
- 4 **100 µl** Stopplösung zugeben
→ Platte **5 Minuten** stehenlassen
→ Bei **450 nm** messen