Code No. 27158

Human Osteopontin Assay Kit - IBL

INTRODUCTION

Osteopontin (OPN) is a secreted glycoprotein that was originally isolated from bone. At present, it is known as a highly acidic calcium-binding glycosylated phosphoprotein secreted by many cell types, including osteoblasts, kidney tubule cells, macrophages, activated T cells, and vascular smooth muscle cells. Its molecular weights have been reported in the range of 66 kDa to 44 kDa depending on glycosylation and phosphorylation.

One important feature of OPN is that it contains an Arg-Gly-Asp (RGD) amino acid sequence. This motif is present in fibronectin, vitronectin and a variety of other extra cellular proteins that bind members of the integrin family of cell surface receptors such as $\alpha v\beta 3$.

Another important of OPN is the presence of various molecular forms in vivo due to differential RNA splicing, glycosylation, phosphorylation, sulfation, and susceptibility to proteases. Both OPN and thrombin are likely to be localized together at the site of injury, inflammation, and angiogenesis and in tumor tissues. Osteopontin is susceptible to proteolytic fragmentation, and this process may have physiologic importance. A report demonstrated that thrombin treatment enhanced OPN cell adhesive activity, suggesting that cleavage of OPN by thrombin exposes a cryptic adhesive sequence. More recently, it was shown that an amino terminal OPN fragment contains a cryptic binding site that can be recognized by $\alpha 9\beta 1$ integrin. Furthermore, OPN contains multiple cell binding sites and interacts with various receptors; these interactions may have distinct functional.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Human OPN.

The epitope of used antibodies are the followings.

Coating Antibody : Anti-Human OPN (O-17) Rabbit IgG Affinity Purify: The antibody reacts at part of N-terminal of human OPN (IPVKQADSGSSEEKQ).

Labeled Antibody : Anti-Human OPN (10A16) Mouse IgG MoAb Fab'-HRP: The antibody reacts at part of the right side from thrombin cleavage site of human OPN (KSKKFRRPDIQYPDATDE).

MEASUREMENT RANGE

5 ~ 320 ng/mL (76.9 ~ 4,920 pmol/L)

INTENDED USE

- This kit is to be used for the in-vitro quantitative determination of Human Osteopontin (Human OPN) in EDTA plasma, urine, or cell culture media. Please store all samples at -80℃ before use because OPN molecule is unstable protein. Since measured value falls by being left in room temperature or repetition of freeze/thaw, cautions are required.
- The recommend dilution for human EDTA plasma samples is about 5 - 10 fold by EIA buffer or PBS. Please assay again with more dilution if the assay with dilution of 5 - 10 fold take range over the high standard value.
- The assay by serum or heparin plasma samples give any values, but it might be not reflected correct values, because OPN is unstable and is easily cleaved by thrombin. And, OPN has several heparin binding sites in the molecules, so that heparin plasma will give any effect in the assay.
- The recommend dilution for urine samples is about more than 200 fold by EIA buffer or PBS, but the dilution rate should be optimized by each laboratories. Since it is easy to decompose a urine sample, we recommend to add PMSF (protease inhibitor) etc. Moreover, when it cannot measure immediately after extraction, please store at -80℃ or less. Since measured value falls by repetition of freeze/thaw, cautions are required. The amount of Human-OPN in urine has report of being in inverse proportion to urine volume. We recommend to carry out creatinine compensation in the case of measurement.
- The recommend dilution for cell culture media samples is various by using cells, therefore, the dilution rate should be optimized by each laboratories.
- The kit can not assay thrombin-cleaved Human OPN.
- Both recombinant and native forms of Human OPN can be detected with the kit.

KIT COMPONENT

1	Precoated plate	: Anti-Human OPN (O-17) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: HRP conjugated Anti-Human OPN (10A16) Mouse IgG MoAb Fab' Affinity Purify (X30)	0.4mL x 1
3	Standard	: Recombinant Human OPN	0.5mL x 2
4	EIA buffer	: 1% BSA, 0.05% Tween 20 in PBS	30mL x 1
5	Solution for Labeled antibody	: 1% BSA, 0.05%Tween 20 in PBS	12mL x 1
6	Chromogen	: TMB solution	15mL x 1
7	Stop solution	: 1N H_2SO_4	12mL x 1
8	Wash buffer Conc.	: 0.05% Tween20 in phosphate buffer (X40)	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Distilled water
- Incubator (37℃±1℃)
- Refrigerator(as 4℃)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- PBS

2. Preparation

1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (X40) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of distilled water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.

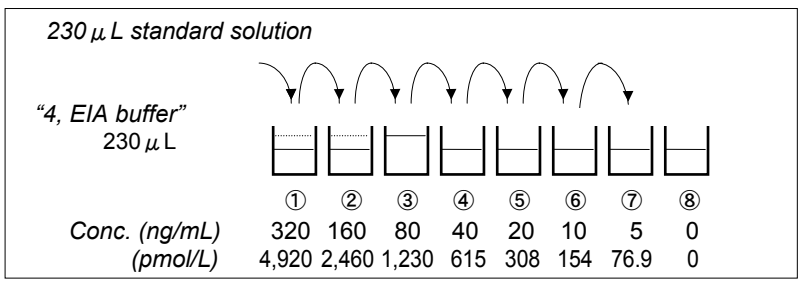
2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (X30). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800 μL. (Dilute 30 μL of "2, Labeled antibody Conc." with 870 μL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μL in each well.)
<u>This operation should be done just before the application of Labeled antibody.</u>
The remaining "2, Labeled antibody Conc." should be stored at 4℃ in firmly sealed vial.

3) Preparation of Standard
Put just 0.5mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is Human OPN standard 640 ng/mL (9,850 pmol/L).

4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube.

Tube-1	320 ng/mL	(4,920 pmol/L)
Tube-2	160 ng/mL	(2,460 pmol/L)
Tube-3	80 ng/mL	(1,230 pmol/L)
Tube-4	40 ng/mL	(615 pmol/L)
Tube-5	20 ng/mL	(308 pmol/L)
Tube-6	10 ng/mL	(154 pmol/L)
Tube-7	5 ng/mL	(76.9 pmol/L)
Tube-8	0 ng/mL	(Test Sample Blank)

Put 230 μL of Standard solution into tube–1 and mix it gently. Then, put 230 μL of tube-1mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 320 ng/mL (4,920 pmol/L) and 5 ng/mL (76.9 pmol/L). Tube-8 is the test sample blank as 0 ng/mL.
See following picture.



5) Dilution of test sample
Test sample may be diluted with "4, EIA buffer" or PBS if the need arises.
Example) Plasma (EDTA) : X5 ~ X10 , Urine : More than X200
If the concentration of Human OPN in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μL	Diluted standard (Tube 1~7) 100 μL	EIA buffer (Tube-8) 100 μL	EIA buffer 100 μL
Incubation for 1 hour at 37℃ with plate lid				
Washing 7 times				
Labeled Antibody	100 μL	100 μL	100 μL	-
Incubation for 30minutes at 4℃ with plate lid				
Washing 9 times				
Chromogen	100 μL	100 μL	100 μL	100 μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μL	100 μL	100 μL	100 μL
Read the plate at 450nm within 30 minutes after application of Stop solution.				

1) Determine wells for reagent blank. Put 100 μL each of "4, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
3) Incubate the precoated plate for 1 hour at 37℃ after covering it with plate lid.
4) Wash each well of the precoated plate vigorously with Wash buffer using washing bottle. Then, fill each well with Wash buffer and place the precoated plate for 15~30 seconds. Remove Wash buffer completely from the precoated plate by snapping.
<u>This procedure must be repeated more than 7 times.</u>
Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
5) Pipette 100 μL of Labeled antibody into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4℃ after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner above 4).
8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 μL from the test tube into the wells. Please avoid to return the rest of test tube into "6, Chromogen" bottle due to avoid to cause of contamination.

Immuno-Biological Laboratories Co., Ltd.
TEL: +81 (0)27-310-8040 FAX : +81 (0)27-310-8045
5-1 Aramachi, Takasaki-shi, Gunma, 370-0831, JAPAN
URL: ***http://www.ibl-japan.co.jp*** ***E-mail:*** ***do-ibl@ibl-japan.co.jp***

9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
10) Pipette 100μL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm. The measurement shall be done within 30 minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

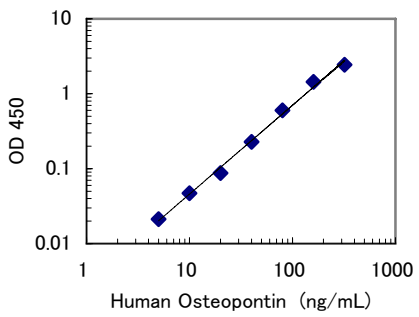
1. Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2. Test samples should be diluted with "4, EIA buffer" or PBS, if the need arises. If the concentration of Human OPN in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.
3. The measurement of test samples and standard in duplicate is recommended.
4. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5. Use only Wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6. Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
7. "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8. Measurement should be done within 30 minutes after addition of "7, Stop solution".
9. Adding PMSF (protease inhibitor) to urine sample is recommended to avoid cleavage of OPN. Moreover, when it cannot measure immediately after collection, please store at -80℃ or less. Since measured value falls by repetition of freeze/thaw, cautions are required.
10. Please perform plasma by EDTA blood collecting. Moreover, when it cannot measure immediately after collection, please store at -80℃ or less. Since measured value falls by repetition of freeze/thaw, cautions are required.

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. ng/mL (pmol/L)	Absorbance (450nm)
320 (4,920)	2.512
160 (2,460)	1.520
80 (1,230)	0.677
40 (615)	0.304
20 (308)	0.164
10 (154)	0.123
5 (76.9)	0.097
0 (Test Sample Blank)	0.076



＊ The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
Human Plasma (EDTA)	5	149.46	144.30	103.6
	10	72.77	74.77	97.3
	20	34.61	40.88	84.7
	40	21.63	25.41	85.1
Human Urine	200	278.74	273.11	102.1
	400	144.15	127.43	113.1
	800	62.39	57.21	109.1
	1,600	26.08	28.76	90.7
10%FCS added TIL media※	80	175.99	162.01	108.6
	160	83.85	74.96	111.9
	320	37.03	35.23	105.1
	640	13.51	13.74	98.4

※TIL Media : Immuno-Biological Laboratories Co., Ltd. Code No.33612

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
Human Plasma (EDTA) (x5)	106.96	116.28	108.7
	86.96	90.43	104.0
	76.96	74.08	96.3
Human Urine (x200)	240.51	240.36	99.9
	220.51	212.04	96.2
	210.51	201.34	95.6
10%FCS added TIL media※ (x80)	117.81	121.69	103.3
	107.81	108.99	101.1
	102.81	101.00	98.2

※TIL Media : Immuno-Biological Laboratories Co., Ltd. Code No.33612

3. Inter – Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
63.90	3.03	4.7	23
35.71	1.58	4.4	23
19.17	1.94	10.1	23

4. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
62.97	5.03	8.0	47
34.90	2.92	8.4	47
18.63	2.04	11.0	47

5. Specificity

Compound	Cross Reactivity
human-OPN	100.0%
mouse-OPN	0.2%
rat-OPN	≦0.1%

6. Sensitivity

3.33 ng/mL (51.2 pmol/L)
The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1 All reagents should be stored at 2~8℃. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to contact your skin and clothes with "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. "1, Precoated plate" and "3, Standard" contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid the production of explosive metallic azide.
5. The precipitation may grow in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 ~ 8℃
The term of validity : 12 months
(The expiry date is specified in outer box.)

REFERENCES

1. Kim J-H., Sakates S. J., Uede T, Wong K-K., Schorge J. O., Feltmate C. M., Berkowitz R. S., Cramer D. W. and Mok S. C. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA 287 (13): 1671-1679, 2002.
2. Yoshitake H., Rittling S. R., Denhardt D. T., and Noda M. Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. Proc. Natl. Acad. Sci. USA. 96: 8156-8160, 1999.
3. Shijubo N., Uede T., Kon S., Nagata M., Abe S. : Vascular endothelial growth factor and osteopontin in tumor biology. Crit Rev Oncog. 11 (2): 135-146, 2000. Review.
4. Chiba S., Rashid M. M., Okamoto H., Shiraiwa H., Kon S., Maeda M., Murakami M., Inobe M., Kitabatake A., Chambers A. F., and Uede T. The role of osteopontin in the development of granulomatous lesions in lung. Microbiol. Immunol. 44 (4): 319-332, 2000.
5. Kon S, Maeda M, Segawa T, Hagiwara Y, Horikoshi Y, Chikuma S, Tanaka K, Rashid MM, Inobe M, Chambers AF, Uede T. : Antibodies to different peptides in osteopontin reveal complexities in the various secreted forms. J. Cell. Biochem. 77 (3): 487-498, 2000.
6. Takemoto M., Yokote K., Nishimura M., Shigematsu T., Hasegawa T., Kon S., Uede T., Matsumoto T., Saito Y., and Mori S. Enhanced expression of osteopontin in human diabetic artery and analysis of its functional role in accelerated atherogenesis. Arterioscler Thromb. Vasc. Biol. 20 (3): 624-628, 2000.
7. Kon S., Maeda M., Segawa T., Hagiwara Y., Horikoshi Y., Chikuma S., Tanaka K., Rashid M. M., Inobe M., Chambers A. F. and Uede T. Antibodies to different peptides in osteopontin reveal complexities in the various secreted forms. J. Cell. Biochem. 77: 487-498, 2000.
8. Weiss J. M, Renkl A. C., Maier C. S., Kimmig M., liaw L., Ahrens T., Kon S., Maeda M., Hotta H., Uede T., and Simon J. C. Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and Dendritic cell migration to lymph nodes., J. Exp. Med.194:1219-1229, 2001.
9. Takahashi F., Takahashi K., Okazaki T., Maeda K., Ienaga H., Maeda M., Kon S., Uede T., Fukuchi Y. Role of osteopontin in the pathogenesis of bleomycin- induced pulmonary fibrosis. Am. J. Respir. Cell. Mol. Biol. 24 (3): 264-271, 2001.
10. Gang X., Ueki K., Kon S., Maeda M., Naruse T., Nojima Y. Reduced urinary excretion of intact osteopontin in patients with IgA nephropathy. Am. J. Kidney Dis. 37 (2):374-379, 2001.
11. Ohshima S., Yamaguchi N., Nishioka K., Mima T., Ishii T., Umeshita-Sasai M., Kobayashi H., Shimizu M., Katada Y., Wakitani S., Murata N., Nomura S., Matsuno H., Katayama R., Kon S., Inobe M., Uede T., Kawase I., and Saeki Y. Enhanced local production of osteopontin in rheumatoid joints. J Rheumatol. 29 (10): 2061-2067, 2002.
12. Ohshima S, Kobayashi H, Yamaguchi N, Nishioka K, Umeshita-Sasai M, Mima T, Nomura S, Kon S, Inobe M, Uede T, Saeki Y. Expression of osteopontin at sites of bone erosion in a murine experimental arthritis model of collagen-induced arthritis: possible involvement of osteopontin in bone destruction in arthritis. Arthritis Rheum. 46 (4): 1094-101, 2002.
13. Kon S., Yokosaki Y., Maeda M., Segawa T., Horikoshi Y., Tsukagoshi H., Rashid M. M., Morimoto J., Inobe M., Shijubo N., Chambers A. F., and Uede T. Mapping of functional epitopes of osteopontin by monoclonal antibodies raised against defined internal sequences. J. Cell. Biochem. 84: 420-432, 2002.
14. Shijubo N., Uede T., Kon S., Maeda M., Segawa T., Imada A., Hirasawa M., and Abe S. : Vascular endothelial growth factor and osteopontin in stage I lung adenocarcinoma. Am. J. Respir. Crit. Care. Med. 160 (4): 1269-1273, 1999.
15. Yumoto K., Ishijima M., Rittling S. R., Tsuji K., Tsuchiya Y., Kon S., Nifuji A., Uede T., Denhardt D. T., and Noda M. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. Proc. Natl. Acad. Sci. USA. 99 (7): 4556-4561, 2002.
16. Koguchi Y., Kawakami K., Kon S., Segawa T., Maeda M., Uede T., and Saito A. Penicillium marneffei causes osteopontin-mediated production of interleukin-12 by peripheral blood mononuclear cells. Infect. Immun. 70 (3), 1042-1048, 2002.

Version
040901 Established
060519 Revised
060807 Revised
061124 Revised

Immuno-Biological Laboratories Co., Ltd.
TEL: +81 (0)27-310-8040 FAX : +81 (0)27-310-8045
5-1 Aramachi, Takasaki-shi, Gunma, 370-0831, JAPAN
URL: http://www.ibl-japan.co.jp ***E-mail:*** do-ibl@ibl-japan.co.jp

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International B.V. Zutphenseweg 55, 7418 AH Deventer, The Netherlands	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

Symbols Version 3.5 / 2010-11-01