Code No. 27360

Rat 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 αvβ3.
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, in- flammation, 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. And then, it was shown that an aminoterminal OPN fragment contains a cryptic binding site that can be recognized by α9β1 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 a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Rat OPN.

MEASUREMENT RANGE

0.07 - 4.75 ng/mL

INTENDED USE

- This kit is to be used for the in-vitro quantitative determination of Rat Osteopontin (Rat OPN) in plasma (EDTA), urine, or cell culture media. Please store all samples at –80 °C before use because OPN molecule is unstable protein.
- The recommend dilution for rat EDTA plasma samples is more than 10 fold by PBS. Please assay again with more dilution if the assay with dilution of more than 10 fold take range over the high standard value.
- The assay by serum or heparin plasma samples are discouraged, because OPN is easily cleaved by thrombin and has several heparin binding sites in the molecule. Therefore, serum won't give correct value and heparin plasma will give any effect in the assay.
- The recommended dilution for urine sample varies by strain of rat, therefore, the dilution rate should be optimized by each laboratory. In some strains, there are almost no full-length molecule of OPN in urine and could not detect with this ELISA. Since OPN in urine sample is easy to be degraded, we recommend adding some protease inhibitor such as PMSF. Moreover, when it cannot measure immediately after collection, please store at -80 °C or less. Since measured value falls by repetition of freeze/thaw cycles, cautions are required.
- The recommend dilution for cell culture media samples is various by expression level of OPN, therefore, the dilution rate should be optimized by each laboratories.
- The kit can not assay thrombin-cleaved Rat OPN.
  Both recombinant and native forms of Rat OPN can be detected with the kit.

KIT COMPONENT

1	Precoated plate	: Anti-Rat OPN (O-17) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc.	: (30X) HRP conjugated Anti- Rat OPN (O-165) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard	: Recombinant Rat OPN	0.5mL x 2
4	EIA buffer	: 1% BSA, 0.05% Tween20 in PBS	30mL x 1
5	Solution for Labeled antibody	: 1% BSA, 0.05% Tween20 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.	: (40X) 0.05% Tween20 in phosphate buffer	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

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

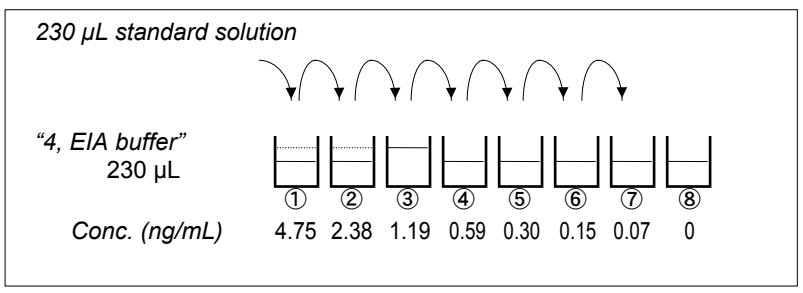
2. Preparation

1) Preparation of wash buffer
   "8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized 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 (30X). 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 strip (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.)
   This operation should be done just before the application of Labeled antibody.
   The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
3) Preparation of Standard
   Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 9.5 ng/mL Rat OPN standard.
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	4.75 ng/mL
Tube-2	2.38 ng/mL
Tube-3	1.19 ng/mL
Tube-4	0.59 ng/mL
Tube-5	0.30 ng/mL
Tube-6	0.15 ng/mL
Tube-7	0.07 ng/mL
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-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 4.75ng/mL and 0.07 ng/mL. 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 as necessary.
   If the concentration of Rat 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. Make sure of 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 60 minutes at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C 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 against a Reagent Blank within 30 minutes after addition 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 60 minutes at 37°C after covering it with plate lid.
4) Wash each well of the precoated plate vigorously with wash buffer using the washing bottle. Then, fill each well with wash buffer and leave the precoated plate laid for 15-30 seconds. Remove wash buffer completely from the precoated plate by snapping. This procedure must be repeated more than 7 times. Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
   In case of using a 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 solution into the wells of test samples, diluted standard and test sample blank.
6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner as 4).
8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by 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 450 nm against a reagent blank.
    The measurement shall be done within 30 minutes after addition of "7, Stop solution".

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***

SPECIAL ATTENTION

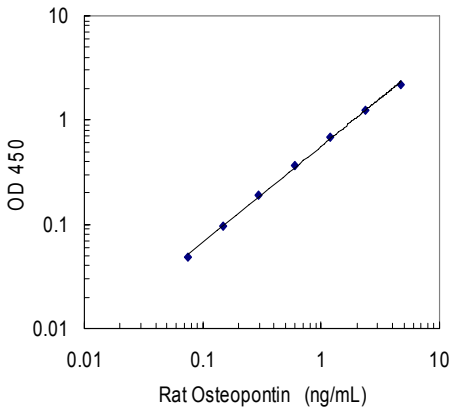
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples should be diluted with “4, EIA buffer” or PBS, if the need arises.
3) Duplicate measurement of test samples and standard 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 some protease inhibitor such as PMSF to urine samples is recommended to avoid cleavage of OPN. Moreover, when it cannot measure immediately after collection, please store at -80 °C or less. Since measured value falls by repetition of freeze/thaw cycles, cautions are required.
10) Please perform plasma by EDTA blood collecting. Moreover, when it cannot measure immediately after collection, please store at -80°C or less. Since measured value falls by repetition of freeze/thaw cycles, 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)	Absorbance (450nm)
4.75	2.237
2.38	1.273
1.19	0.692
0.59	0.379
0.30	0.207
0.15	0.111
0.07	0.063
0 (Test Sample Blank)	0.015



＊ 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 (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	%
10%FCS added RPMI-1640	2	1.11	1.19	93.3
	4	0.59	0.60	98.3
	8	0.30	0.30	100.0
Rat Plasma (EDTA)	6	2.72	3.39	80.2
	12	1.78	1.71	104.1
	24	0.93	0.89	104.5
Rat Urine	1,000	1.75	1.59	110.1
	2,000	0.86	0.79	108.9
	4,000	0.41	0.39	105.1

2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10%FCS added RPMI-1640 (x2)	1.19	0.98	82.4
	0.60	0.53	88.3
	0.30	0.26	86.7
Rat Plasma (EDTA) (x10)	2.76	2.26	81.9
	2.17	2.29	105.5
	1.87	2.15	115.0
Rat Urine (x1,000)	1.60	1.62	101.3
	1.01	1.12	110.9
	0.71	0.78	109.9

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
3.11	0.10	3.2	23
0.72	0.04	5.6	23
0.22	0.01	4.5	23

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
3.18	0.16	5.0	35
0.73	0.04	5.5	35
0.22	0.01	4.5	35

5. Specificity

Compound	Cross Reactivity
Rat Osteopontin	100 %
Human Osteopontin	≦0.1 %
Mouse Osteopontin	≦0.1 %

6. Sensitivity

0.01 ng/mL
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°C. 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 have your skin and clothes contact “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 production of explosive metallic azide.
5. Precipitation may occur 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 a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The term of validity : 12 months
(The expiry date is specified on outer box.)

REFERENCE

1. Kim JH, Skates SJ, Uede T, Wong KK, Schorge JO, Feltmate CM, Berkowitz RS, Cramer DW, Mok SC. Osteopontin as a potential diagnostic biomarker for ovarian cancer. JAMA. 2002 Apr 3;287(13):1671-9.
2. Yoshitake H, Rittling SR, Denhardt DT, Noda M. Osteopontin-deficient mice are resistant to ovariectomy-induced bone resorption. Proc Natl Acad Sci U S A. 1999 Jul 6;96(14):8156-60.
3. Shijubo N, Uede T, Kon S, Nagata M, Abe S. Vascular endothelial growth factor and osteopontin in tumor biology. Crit Rev Oncog. 2000;11(2):135-46.
4. Chiba S, Rashid MM, Okamoto H, Shiraiwa H, Kon S, Maeda M, Murakami M, Inobe M, Kitabatake A, Chambers AF, Uede T. The role of osteopontin in the development of granulomatous lesions in lung. Microbiol Immunol. 2000;44(4):319-32.
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. 2000 Apr;77(3):487-98.
6. Takemoto M, Yokote K, Nishimura M, Shigematsu T, Hasegawa T, Kon S, Uede T, Matsumoto T, Saito Y, Mori S. Enhanced expression of osteopontin in human diabetic artery and analysis of its functional role in accelerated atherogenesis. Arterioscler Thromb Vasc Biol. 2000 Mar;20(3):624-8.
7. Weiss JM, Renkl AC, Maier CS, Kimmig M, Liaw L, Ahrens T, Kon S, Maeda M, Hotta H, Uede T, Simon JC. Osteopontin is involved in the initiation of cutaneous contact hypersensitivity by inducing Langerhans and dendritic cell migration to lymph nodes. J Exp Med. 2001 Nov 5;194(9):1219-29.
8. 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. 2001 Mar;24(3):264-71.
9. 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. 2001 Feb;37(2):374-9.
10. 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, Saeki Y. Enhanced local production of osteopontin in rheumatoid joints. J Rheumatol. 2002 Oct;29(10):2061-7.
11. 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. 2002 Apr;46(4):1094-101.
12. Kon S, Yokosaki Y, Maeda M, Segawa T, Horikoshi Y, Tsukagoshi H, Rashid MM, Morimoto J, Inobe M, Shijubo N, Chambers AF, Uede T. Mapping of functional epitopes of osteopontin by monoclonal antibodies raised against defined internal sequences. J Cell Biochem. 2002;84(2):420-32.
13. Shijubo N, Uede T, Kon S, Maeda M, Segawa T, Imada A, Hirasawa M, Abe S. Vascular endothelial growth factor and osteopontin in stage I lung adenocarcinoma. Am J Respir Crit Care Med. 1999 Oct;160(4):1269-73.
14. Yumoto K, Ishijima M, Rittling SR, Tsuji K, Tsuchiya Y, Kon S, Nifuji A, Uede T, Denhardt DT, Noda M. Osteopontin deficiency protects joints against destruction in anti-type II collagen antibody-induced arthritis in mice. Proc Natl Acad Sci U S A. 2002 Apr 2;99(7):4556-61.
15. Koguchi Y, Kawakami K, Kon S, Segawa T, Maeda M, Uede T, Saito A. Penicillium marneffei causes osteopontin-mediated production of interleukin-12 by peripheral blood mononuclear cells. Infect Immun. 2002 Mar;70(3):1042-8.

Version 1.

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