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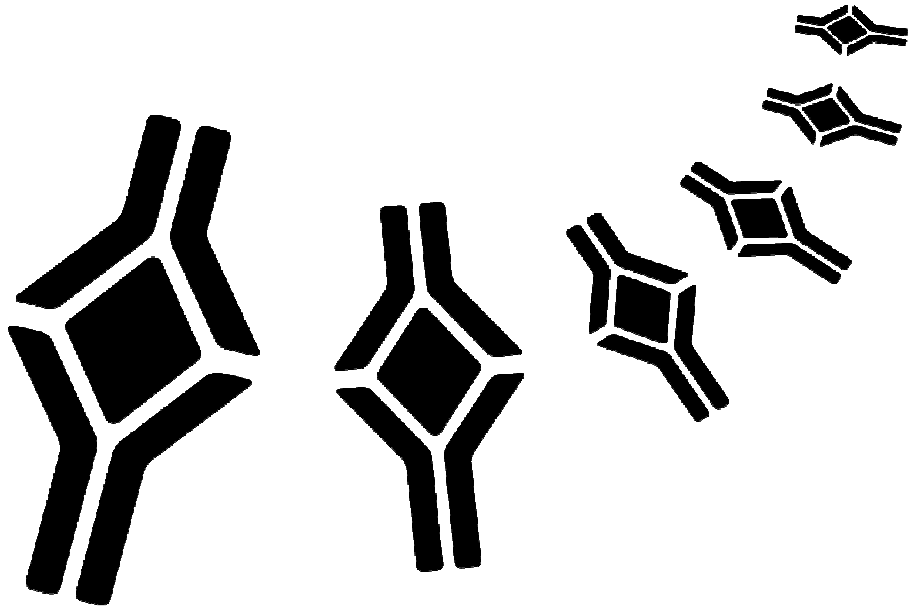


HUMAN ANGIOTENSIN-CONVERTING ENZYME (ACE) ELISA

Product Data Sheet

Cat. No.: BBT0557R

For Research Use Only

VERSION 51 150311 03

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

For quantitative detection of human ACE in sera, plasma, body fluids, tissue lysates or cell culture supernates.

2. STORAGE, EXPIRATION

Storage
Store at 4°C for frequent use, at -20°C for infrequent use.
Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration
Four months at 4°C and eight months at -20°C.

3. INTRODUCTION

Angiotensin-converting enzyme (ACE) is a zinc-containing dipeptidyl carboxypeptidase widely distributed in mammalian tissues and is thought to play a critical role in blood pressure regulation. The predicted protein is identical, from residue 37 to its C terminus, to the second half or C-terminal domain of the endothelial ACE sequence. The protein sequence inferred consists of a 732-residue preprotein including a 31-residue signal peptide. The mature polypeptide has a molecular weight of 80,073.[1] Although ACE has been studied primarily in the context of its role in blood pressure regulation, this widely distributed enzyme has many other physiological functions. The ACE gene encodes two isozymes. The somatic isozyme is expressed in many tissues, including vascular endothelial cells, renal epithelial cells, and testicular Leydig cells, whereas the testicular or germinal angiotensin-converting enzyme is expressed only in sperm.[2] The standard product used in this kit is recombinant human ACE, consisting of 30-1261 amino acids with the molecular mass of 120KDa.

4. TEST PRINCIPLE

Biovendor's Human ACE ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human ACE specific-specific polyclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human ACE amount of sample captured in plate.

5. PRECAUTIONS

- Before using Kit, spin tubes and bring down all components to bottom of tube.
- Duplicate well assay was recommended for both standard and sample testing.
- Don't let 96-well plate dry, dry plate will inactivate active components on plate.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

6. REAGENT SUPPLIED

- Lyophilized recombinant human ACE standard: 10 ng/tube×2.
- One 96-well plate precoated with anti- human ACE antibody.
- Sample diluent buffer: 30 ml
- Biotinylated anti- human ACE antibody : 130 µl, dilution 1:100.
- Antibody diluent buffer: 12 ml.
- Avidin-Biotin-Peroxidase Complex (ABC) : 130 µl, dilution 1:100.
- ABC diluent buffer: 12 ml.
- TMB color developing agent: 10 ml.
- TMB stop solution: 10 ml.

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Microplate reader in standard size.
- Automated plate washer.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
- Clean tubes and Eppendorf tubes.
- Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS:** Add 1.2 g Tris, 8.5 g Nacl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

8. PREPARATION OF REAGENTS AND SAMPLES

Plate Washing

Aspirate the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.4 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes.

Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

Cell culture supernate, tissue lysate or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C

Serum: Allow the serum to clot in a serum separator tube (about 2 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge for 30 min at 1000 x g within 15 min of collection. Analyze immediately or aliquot and store frozen at -20°C. EDTA and citrate are not recommended as the anticoagulant.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice.

Serum/ plasma samples

Dilute samples 100x with Sample diluent buffer just prior to the assay, e.g. 2 μl of sample + 198 μl of Sample diluent buffer for singlets, or preferably 5 μl of sample + 495 μl of Sample diluent buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Serum/plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine samples have to be stored at -70°C. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

The sample must be well mixed with the diluents buffer.

Other type of samples:

High target protein concentration (100-1000 ng/ml). The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.

Medium target protein concentration (10-100 ng/ml). The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.

Low target protein concentration (156-10,000 pg/ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.

Very Low target protein concentration (≤156 pg/ml). No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

- Reconstitution of the human ACE standard□ACE standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of ACE standard (10ng per tube) are included in each kit. Use one tube for each experiment.
  1. 10,000 pg/ml of human ACE standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.
  2. 5000 pg/ml→156 pg/ml of human ACE standard solutions: Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10, 000 pg/ml ACE standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution may be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

- Preparation of biotinylated anti-human ACE antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.
  1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  2. Biotinylated anti-human ACE antibody should be diluted in 1:99 with the antibody diluent buffer and mixed thoroughly.
- Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.
  1. The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
  2. Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:99 with the ABC dilution buffer and mixed thoroughly.

Ask for protocol at info@biovendor.com if assaying tissue culture medium samples.

9. ASSAY PROCEDURE

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard ACE detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of ACE amount in samples.

1. Aliquot 0.1 ml per well of the 10,000 pg/ml, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 313 pg/ml, 156 pg/ml human ACE standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1 ml of each properly diluted sample of human sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human ACE standard solution and each sample is measured in duplicate.
2. Seal the plate with the cover and incubate at 37°C for 90 min.
3. Remove the cover, aspirate plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 0.1 ml of biotinylated anti-human ACE antibody working solution into each well and incubate the plate at 37°C for 60 min.
5. Wash the plate three times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Aspirate the washing buffer and blot the plate onto paper towels or other absorbent material.
6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.
7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Aspirate the washing buffer and blot the plate onto paper towels or other absorbent material.

8. Add 90 µl of prepared TMB color developing agent into each well and incubate plate at 37°C for 21-25 min (shades of blue can be seen in the wells with the four most concentrated human ACE standard solutions; the other wells show no obvious color).
9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human ACE concentration of the samples can be interpolated from the standard curve.
Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

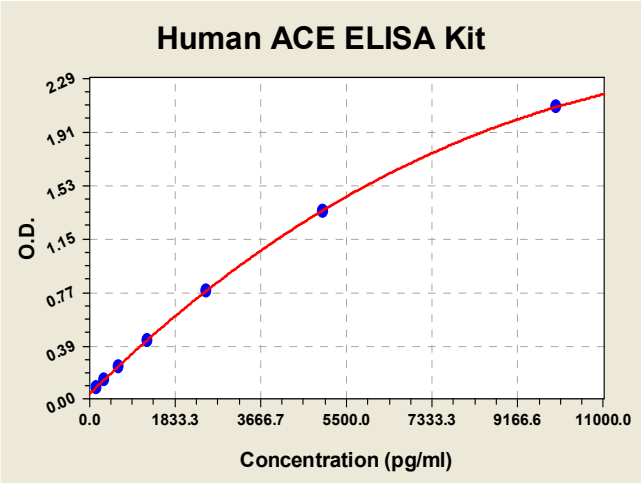
Summary
1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C for 21-25 min.
5. Add TMB stop solution and read.

10. CALCULATIONS

Typical Data Obtained from Human ACE

Concentration	0.0 pg/ml	156 pg/ml	313 pg/ml	625 pg/ml	1250 pg/ml	2500 pg/ml	5000 pg/ml	10,000 pg/ml
O.D	0.044	0.094	0.152	0.243	0.431	0.780	1.353	2.088

Typical Human ACE ELISA Kit Standard Curve
This standard curve was generated at BioVendor for demonstration purpose only. A standard curve must be run with each assay.



11. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human ACE ELISA are presented in this chapter.

- **Sensitivity**

< 60 pg/ml

- **Specificity**

No detectable cross-reactivity with any other cytokine.

- **Range**

156 pg/ml-10,000 pg/ml

- **Precision**

Intra-assay (Within-Run, n=8)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	102.2	2.25	2.2
2	104.0	1.98	1.9

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

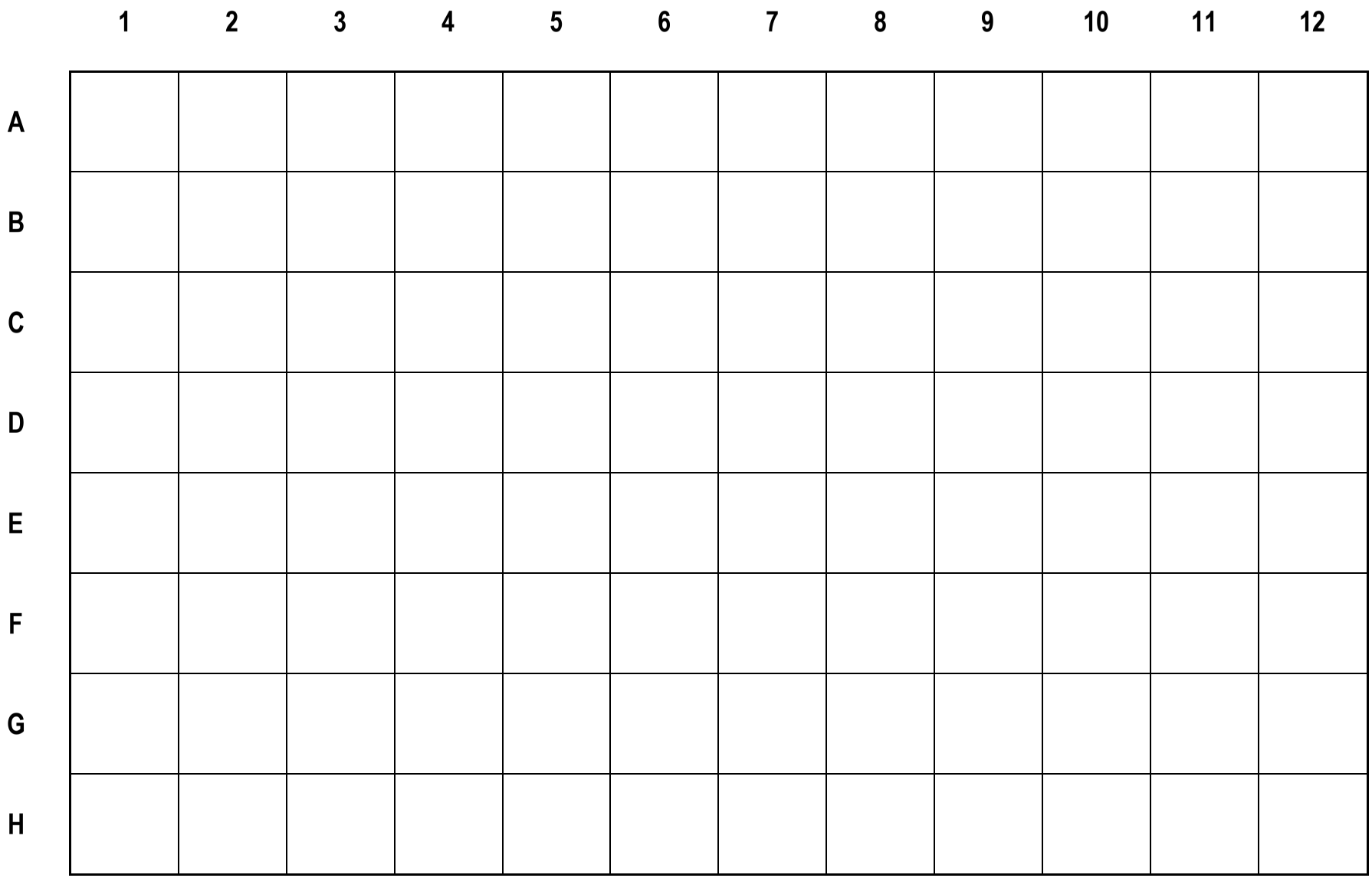
Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	- 2x	54.3 27.8	- 27.1	- 102.2
2	- 2x	77.1 44.2	- 38.5	- 114.6

12. REFERENCES

References to human ACE:

1. Ehlers, M. R. W.; Fox, E. A.; Strydom, D. J.; Riordan, J. F. Molecular cloning of human testicular angiotensin-converting enzyme: the testis isozyme is identical to the C-terminal half of endothelial angiotensin-converting enzyme. *Proc. Nat. Acad. Sci.* 86: 7741-7745, 1989.
2. Ramaraj, P.; Kessler, S. P.; Colmenares, C.; Sen, G. C. Selective restoration of male fertility in mice lacking angiotensin-converting enzymes by sperm-specific expression of the testicular isozyme. *J. Clin. Invest.* 102: 371-378, 1998.

» For more references on this product see our WebPages at www.biovendor.com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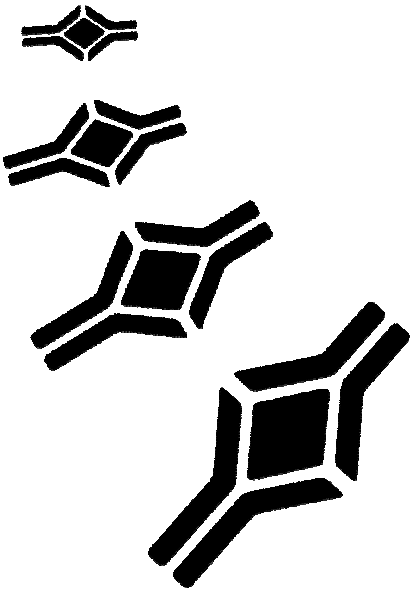

HEADQUARTERS: **BioVendor Laboratorní medicína a.s.**	**Karasek 1767/1**	**621 00 Brno** **CZECH REPUBLIC**	**Phone: +420-549-124-185** **Fax: +420-549-211-460**	**E-mail: info@biovendor.com** **Web: www.biovendor.com**
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	1463 Sand Hill Road Suite 227	Candler, NC 28715 USA	Phone: +1-828-670-7807 +1-800-404-7807 Fax: +1-828-670-7809	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2917, 29/F R & F Ying Feng Plaza, No.2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-38065519 Fax: +86-20-38065529	E-mail: infoCN@biovendor.com