

# ImmunoCAP<sup>®</sup> ISAC

Directions for Use

ImmunoCAP ISAC<sup>®</sup> Assay Kit IgE

ImmunoCAP ISAC<sup>®</sup> Starter Kit IgE

For Research Use Only

20-01-02-3-EN-RUO

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**Bibliography**

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**Developed by:**

Phadia Multiplexing Diagnostics GmbH, Wien, Austria, and

Phadia AB, Uppsala, Sweden

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## INTENDED USE

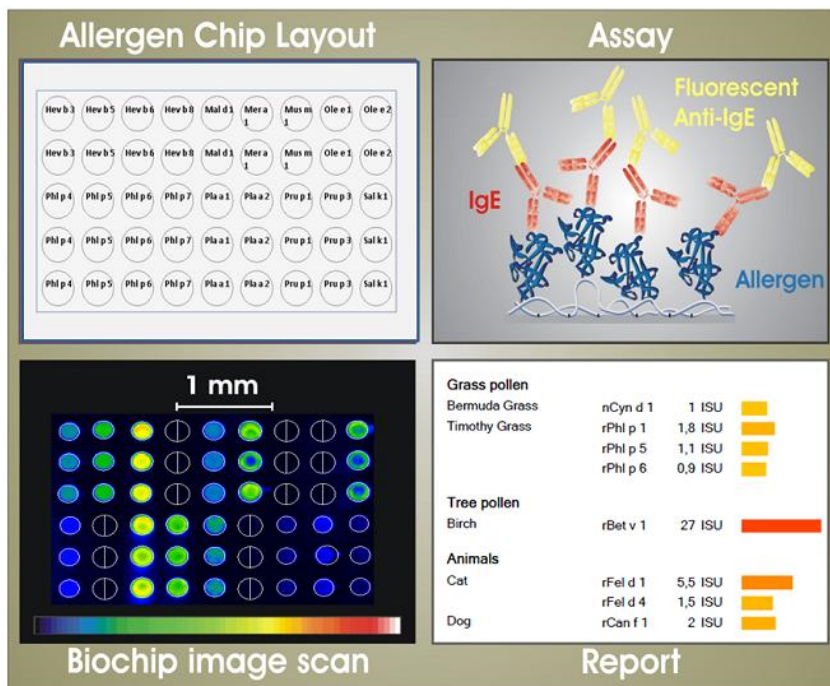
ImmunoCAP ISAC IgE is an *in vitro* test for semi-quantitative determination of specific IgE antibodies in human serum or plasma. It is intended for Research Use Only.

## SUMMARY AND EXPLANATION OF THE TEST

Immediate type of allergic reactions is a function of antibodies belonging to the IgE class of immunoglobulins. Clinical manifestations such as asthma, hay fever, atopic eczema and gastro intestinal symptoms develop after exposure to specific allergens (1). Determination of the sensitization pattern to specific and/or cross reactive allergen components assists in a more detailed evaluation of the allergic patient (2-12).

## PRINCIPLES OF THE PROCEDURE

ImmunoCAP ISAC IgE is a solid-phase immunoassay. Allergen components that are immobilized on a solid substrate in a microarray format are incubated with human serum or plasma samples to detect specific IgE antibodies. Binding of the specific IgE antibodies to the immobilized allergen components is detected by the addition of a secondary fluorescence-labeled anti-human IgE antibody. The procedure is followed by image acquisition using an appropriate microarray scanner. The ISAC Standardized Units for specific IgE (ISU) are determined and the test results are analyzed with proprietary software (MIA - Microarray Image Analysis Software).



**Figure 1.**

**Top left:** Schematic of allergen component microarray layout.

**Top right:** Schematic of assay principle. Allergen components are covalently coupled to the solid phase. Allergen specific IgE antibodies are detected by fluorescence-labeled anti-IgE.

**Bottom left:** Scan image of fluorescence laser scanning microscope showing different intensities from black (negative) to white (strong positive) on false color scale.

**Bottom right:** Schematic of ImmunoCAP ISAC Assay Kit IgE assay reporting.

## REAGENTS

ImmunoCAP ISAC Assay Kit IgE and ImmunoCAP ISAC Starter Kit IgE contain the reagents required to perform an assay. The expiration date and storage temperature for each kit is stated on the outer label. However, each component is stable until the date stated on each individual component's label.

It is not recommended to pool any reagents.

### ImmunoCAP ISAC Starter Kit IgE (Art No 81-1001-01)

(for 20 determinations)

ImmunoCAP ISAC IgE including 4 reaction sites	5 chips	Store at 2-8°C until expiration date. Allow to reach ambient temperature before opening vacuum seal. After vacuum seal is broken chips can be stored for 6 weeks if kept dry and dark at 18 – 32°C. Do not reuse.
Component A (20 x Buffer), 200 ml	1 bottle	Store at 2 - 8°C until expiration date.
IgE Detection Antibody (Fluorescence conjugated anti-human IgE), 0,5 ml	1 vial	Ready for use. 20 determinations. Store at 2 - 8°C until expiration date. Store protected from light.
IgE Control Serum (human serum). 50 µl Sodium azide <0,1%	1 vial	Ready for use. 1 determination. <b>Do not dilute control serum !</b> Store at 2 - 8°C until expiration date.
Washing Dish	2 dishes for 10 chips	Ready for use.
Magnetic stirrer bar	2	Ready for use.
Humidity Chamber	1 chamber for 26 chips	Ready for use.

## ImmunoCAP ISAC Assay Kit IgE (Art No 81-1000-01)

(for 20 determinations)

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IgE Detection Antibody (Fluorescence conjugated anti-human IgE), 0,5 ml	1 vial	Ready for use. 20 determinations. Store at 2 - 8°C until expiration date. Store protected from light.
IgE Control Serum (human serum), 50 µl Sodium azide <0,1%	1 vial	Ready for use. 1 determination. <b>Do not dilute control serum !</b> Store at 2 - 8°C until expiration date.

### Other Materials

Materials required but not provided by Phadia AB:

- Measuring cylinder 1000 ml
- Purified water
- Magnetic stirrer

### PRECAUTIONS

- For Research Use Only. Not for internal or external use in humans or animals.
- Do not use reagents beyond their expiration dates.
- It is recommended to wear hand and eye protection and follow good laboratory practices when preparing and handling reagents and samples.
- This kit contains reagents manufactured from human blood components. The source materials have been tested negative by immunoassay for hepatitis B surface antigen and for antibodies to HIV1, HIV2 and hepatitis C virus. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or other local/national guidelines on laboratory safety procedures.
- **Warning!** Reagents that contain sodium azide as a preservative must be handled with care. Safety Data Sheet available from Phadia AB on request.

## **Handling of ImmunoCAP ISAC**

Do not label with pens or markers that are soluble in water or organic solvents. Residuals of labeling can interfere with the fluorescence-based analysis of ImmunoCAP ISAC. If necessary, use a pencil for labeling. Avoid direct contact with the surface of ImmunoCAP ISAC during any of the reaction steps. Always touch ImmunoCAP ISAC at the margin of the glass slide.

## **PREPARATION OF SPECIMEN, REAGENTS AND EQUIPMENT**

### **Specimen**

Serum or plasma samples from venous or capillary blood can be used. Collect blood samples using standard procedures. Keep specimens at room temperature for shipping purposes only. Store at 2 – 8°C for up to one week or at –20°C for prolonged storage. Avoid repeated freezing and thawing.

### **Solution A**

Prepare 700 ml of a fresh 1:20 dilution of Component A in purified water to obtain Solution A (add 665 ml purified water to 35 ml Component A). The volume is calculated for 3 washing steps of 220 ml each, using the Washing Dishes provided with the Starter kit. The volume of the dilution shall be adjusted individually to the container being used.

### **IgE Detection Antibody**

IgE Detection Antibody solution is ready for use. Protect from light and avoid freezing.

### **Humidity Chamber**

Place a fresh paper towel in the bottom of the humidity chamber and soak it with purified water. Until further use, close the lid of the humidity chamber to prevent evaporation.

### **ImmunoCAP ISAC**

Put ImmunoCAP ISAC into the Washing Dish containing the removable glass slide rack (up to 10 chips) and approximately 220 ml of Solution A together with a magnetic stirring bar in the bottom of the dish. Place it onto a magnetic stirrer and stir vigorously for 60 minutes. Move the glass slide rack containing the ImmunoCAP ISAC into a separate Washing Dish containing approximately 220 ml purified water. Add a stirring bar and stir vigorously on a magnetic stirrer for 5 minutes. Remove the glass slide rack containing ImmunoCAP ISAC and place it onto a paper towel and leave to air-dry. Wait until the chips are completely dry. Continue with the Test Procedure immediately afterwards. Discard all used washing solutions.

## TEST PROCEDURE

- Place the prepared ImmunoCAP ISAC IgE in the Humidity Chamber with the reaction sites up.
- Pipette 20  $\mu$ l of each sample onto one reaction site (there are 4 reaction sites available per chip). Close the Humidity Chamber carefully, without mixing the samples. It is recommended to use one IgE Control Serum per ImmunoCAP ISAC Assay Kit IgE. Assay of IgE Control Serum is mandatory if changes to the assay or scanning procedure are introduced. Avoid direct contact of the pipette tip with the surface of ImmunoCAP ISAC IgE when dispensing the sample.
- Incubate at room temperature for 120 minutes.
- Remove ImmunoCAP ISAC IgE from the Humidity Chamber carefully, without mixing the samples. Remove the samples by tapping the chip with its long side on a fresh paper towel. Be careful to shake off the samples at right angles with the glass edge to avoid samples running over neighboring reaction sites.
- Wash ImmunoCAP ISAC IgE with approximately 220 ml Solution A for 10 minutes (using the Washing Dish and the magnetic stirrer, as described previously).
- Move the glass slide rack containing ImmunoCAP ISAC IgE into a Washing Dish containing approximately 220 ml purified water. Wash for 5 minutes.
- Allow the washed ImmunoCAP ISAC IgE to air dry until completely dry.
- ImmunoCAP ISAC IgE is now ready for incubation with IgE Detection Antibody solution. Place the dry ImmunoCAP ISAC IgE in the Humidity Chamber with the reaction sites facing upwards.
- Discard all used washing solutions.
- Pipette 20  $\mu$ l of IgE Detection Antibody solution onto each reaction site of the ImmunoCAP ISAC. Make sure that ImmunoCAP ISAC IgE is properly placed in the Humidity Chamber and close the lid.
- Incubate at room temperature for 60 minutes, protected from light.
- Remove ImmunoCAP ISAC IgE from the Humidity Chamber carefully. Remove IgE Detection Antibody solution by tapping ImmunoCAP ISAC IgE with its long side on a fresh paper towel or rinse gently under distilled water.
- Wash ImmunoCAP ISAC IgE with approximately 220 ml Solution A for 10 minutes (using the Washing Dish and the magnetic stirrer, as described previously).
- Move the glass slide rack containing ImmunoCAP ISAC IgE into a Washing Dish containing approximately 220 ml purified water. Wash for 5 minutes.
- Discard all used washing solutions.
- Allow the washed ImmunoCAP ISAC IgE to air dry until completely dry.
- ImmunoCAP ISAC IgE is now ready for reading. Use it directly for data acquisition in an appropriate microarray scanner or store dry and protected from light for subsequent reading.

### **Parameters of the procedure**

Volumes per determination:

Sample	20 µl
IgE Detection Antibody	20 µl
Solution A	660 ml

Total time for one assay is 5 hours.

Incubations are performed at room temperature.

### **Reference material**

ImmunoCAP ISAC IgE internal calibration is made against an in-house reference serum and measured IgE antibody concentrations are expressed as arbitrary units; ISAC Standardized Units, (ISU). The ImmunoCAP ISAC IgE in house reference serum is standardized against ImmunoCAP Specific IgE.

ImmunoCAP Specific IgE Calibrators are traceable (via an unbroken chain of calibrations) to the 2nd International Reference Preparation (IRP) 75/502 of Human Serum Immunoglobulin E from the World Health Organization (WHO). Subsequently IgE antibody results (ISU) obtained with ImmunoCAP ISAC are indirectly linked to the WHO IRP 75/502 IgE.

### **Measuring Range**

0,3 - 100 ISU.

### **Quality Control**

**Record keeping for each assay:** It is good laboratory practice to record the lot numbers of the components used, the dates when they were first opened and the remaining volumes.

**Control Specimens:** Good laboratory practice requires that quality control specimens should be included in one run per kit.

The provided IgE Control Serum should be run with defined intervals for the system to provide accurate ISU measurements.



## DATA ANALYSIS

### Scanning Procedure

For the analysis of ImmunoCAP ISAC IgE, we recommend the use of confocal laser scanning devices, in particular the CapitalBio LuxScan™ 10K microarray scanner.

### Specifications for the microarray scanner

Chip Format	26 mm × 76 mm
Scan Resolution	10 µm
Sensitivity	0.1 fluorescent molecules/µm <sup>2</sup>
Dynamic Range	16 bit
Maximum Scan Area	22×72 mm
Excitation Wavelength	532 nm and/or 635 nm (Green and Red Laser)
Fluorescent Dyes	Alexa Fluor 532 nm, Alexa Fluor 647 nm
Image File Format	16 bit Grayscale TIFF

A scanning protocol for ImmunoCAP ISAC IgE is set up during system installation by a technical product specialist. In general, images should be acquired with laser power set as recommended by the instrument manufacturer, and all other settings chosen to prevent saturated (out of range) signals on the scan images.

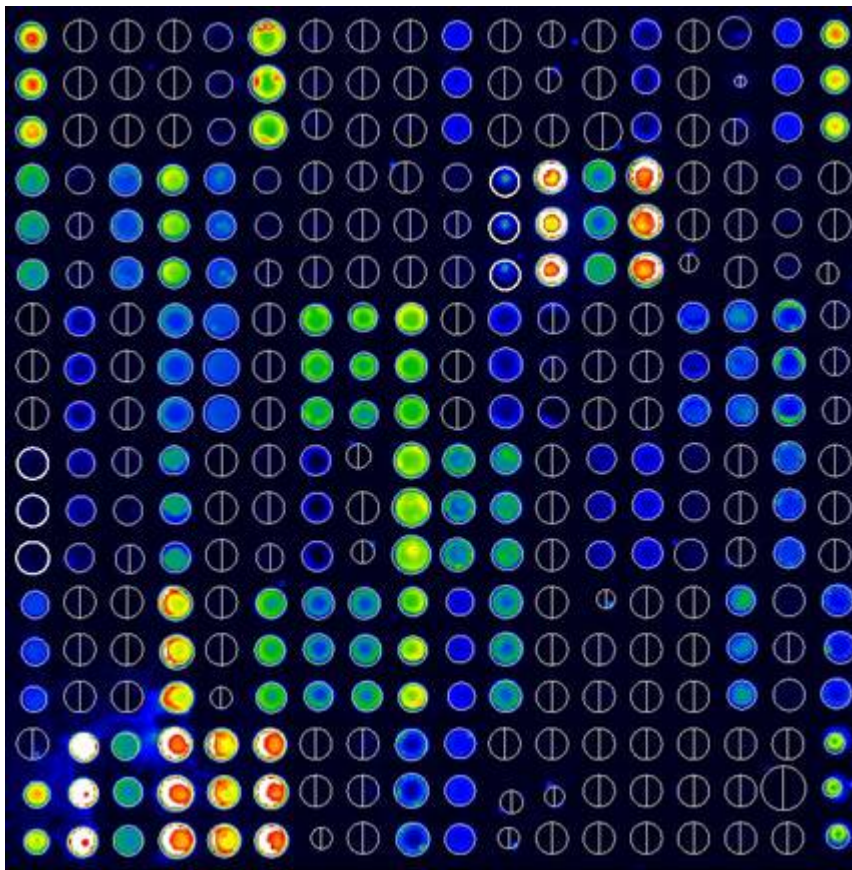
### Image Analysis Procedure

For analyzing ImmunoCAP ISAC IgE, we recommend using MIA – Microarray Image Analyzer software, which is installed during instrument setup by our service specialist. MIA facilitates the automatic analysis of ImmunoCAP ISAC IgE. Chip scan images are analyzed and results are stored in a database as well as directly reported to the user. MIA has an interface to export ImmunoCAP ISAC IgE data to the ImmunoCAP Information Data Manager (IDM).

### Results

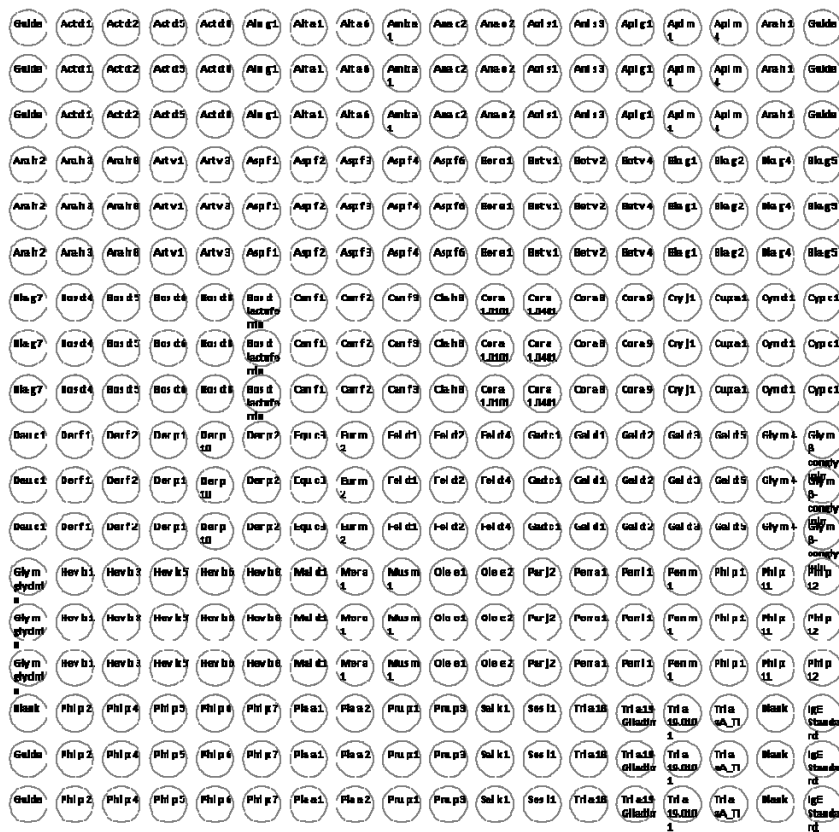
ImmunoCAP ISAC IgE is a semi-quantitative method where allergen component specific IgE antibodies are measured in arbitrary units, ISU (ISAC Standardized Units). The results are presented semi-quantitatively in 4 classes (0 = Undetectable or very low, 1 = Low, 2 = Moderate to High, 3 = Very high). The MIA software automatically performs this calculation.

<b>ImmunoCAP ISAC IgE Range (IgE antibody level)</b>	<b>Corresponds to ISU</b>
0 (Undetectable or Very Low)	< 0.3
1 (Low)	≥ 0.3 - < 1
2 (Moderate to High)	≥ 1 - < 15
3 (Very High)	≥ 15



0  65.535

**Figure 2a.**  
**Example of ImmunoCAP**  
**ISAC image scan.** All  
 allergens are arrayed in  
 vertical triplicates. To visualize  
 the fluorescence output the  
 image is shown in false color  
 display mode.  
 Linear scale of false color  
 display is shown below the  
 image.



**Figure 2b.**  
**ImmunoCAP ISAC array**  
**layout.** Layout shows  
 positions and names of 103  
 allergen components. Layout  
 and allergen panel may be  
 subject to changes.

## **LIMITATIONS OF THE PROCEDURE**

This product is for Research Use Only.

## **EXPECTED VALUES**

The association between allergen specific IgE antibodies and allergic disease is well established and has been extensively documented in the scientific literature (1). Each sensitized patient will have an individual IgE antibody profile when tested in ImmunoCAP ISAC IgE.

When samples from healthy non-allergic blood donors were tested with ImmunoCAP ISAC IgE, the response units for all allergen components were well below 0.3 ISU.

Good laboratory practice recommends that each laboratory establishes its own range of expected values.

## **PERFORMANCE CHARACTERISTICS**

**Limit of Detection:** LoD was determined in accordance with CLSI guideline EP17-A (13) for representative allergen components. The general LoD was estimated to 0,3 ISU.

**Precision:** Precision was determined in accordance with CLSI guideline EP5-A2 (14) for representative allergen components. The within run CV was estimated to 15 % and the total CV was estimated to 25 %.

**Analytical Specificity:** The IgE Detection Antibody does not react with other immunoglobulins in human serum.

The performance characteristics stated are general parameters that can deviate for individual allergen components. Performance characteristics were determined for representative allergen components only and may not be applicable for all allergen components.

## **WARRANTY**

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. Phadia AB in such event shall not be liable for damages indirect or consequential.

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**AUSTRIA Phadia Austria GmbH**

Floridsdorfer Hauptstraße 1, AT-1210 VIENNA  
Tel: +43 1 270 20 20, Fax: +43 1 270 20 20 20

**BELGIUM Phadia NV/SA**

Raketstraat, 64 (2nd floor), BE-1130 BRUSSELS  
Tel: +32 2 749 55 15, Fax: +32 2 749 55 23

**BRAZIL Phadia Diagnósticos Ltda.**

Rua Luigi Galvani, 70 -10º andar - conj. 101  
Cidade Monções - São Paulo - SP Cep: 04575-020  
Tel: +55 11 3345 5050, Fax: +55 11 3345 5060

**DENMARK Phadia ApS**

Gydevang 33, DK-3450 ALLERØD  
Tel: +45 7023 3306, Fax: +45 7023 3307

**FINLAND Phadia Oy**

Rajatorpantie 41 C, FIN-01640 VANTAA  
Tel: +358 9 8520 2560, Fax: +358 9 8520 2565

**FRANCE Phadia SAS**

BP 610, FR-78056 ST QUENTIN YVELINES CEDEX  
Tel: +33 1 6137 3430, Fax: +33 1 3064 6237

**GERMANY Phadia GmbH**

Postfach 1050, DE-79010 FREIBURG  
Tel: +49 761 47805 0, Fax: +49 761 47805 338

**IRELAND Phadia Ltd (Irish Branch)**

Beaghbeg, Carrigallen, LEITRIM  
Tel: +44 1908 84 70 34, Fax: +44 1908 84 75 54

**ITALY Phadia S.r.l.**

Via Libero Temolo, 4, IT-201 26 MILAN  
Tel: +39 02 64 163 411, Fax: +39 02 64 163 415

**JAPAN Phadia K.K.**

Tokyo Opera City Tower, 3-20-2, Nishi-shinjuku Shinjuku-ku, TOKYO 163-1431  
Tel: +81 3 5365 8332, Fax: +81 3 5365 8336

**KOREA Phadia Korea Co. LTD.,**

20 Fl, IT Mirea Tower, 60-21, Gasan-dong Geumcheon-gu, Seoul 153-801  
Tel: +82 2 2027 5400, Fax: +82 2 2027 5404

**THE NETHERLANDS Phadia B.V.**

Postbus 696, NL-3430 AR NIEUWEGEIN  
Tel: +31 30 602 37 00, Fax: +31 30 602 37 09

**NORWAY Phadia AS**

Postboks 4814, Nydalen, NO-0422 OSLO  
Tel: +47 21 67 32 80, Fax: +47 21 67 32 81

**PORTUGAL Phadia Sociedade Unipessoal Lda.**

Lagoas Park - Edifício No. 11 - Piso 0, PT-2740-270 PORTO SALVO  
Tel: +351 21 423 53 50, Fax: +351 21 421 60 36

**SOUTH AFRICA Laboratory Specialities (PTY)**

P.O. Box 1513, Randburg 2125  
Tel: +27 11 793 5337, Fax: +27 11 793 1064

**SPAIN Phadia Spain SL**

Ctra. Rubí 72-74 (Edificio Horizon), ES-08173 Sant Cugat del Vallès, Barcelona  
Tel: +34 935 765 800, Fax: +34 935 765 820

**SWEDEN Phadia AB**

Marknadsbolag Sverige, Box 6460, SE-751 37 UPPSALA  
Tel: +46 18 16 50 00, Fax: +46 18 16 63 24

**SWITZERLAND Phadia AG**

Sennweidstrasse 46, CH-6312 STEINHAUSEN  
Tel: +41 43 343 40 50, Fax: +41 43 343 40 51

**TAIWAN Phadia Taiwan Inc.**

8F.-1, No. 147, Sec. 2, Jianguo N. Rd., Taipei 104 Taiwan R.O.C.  
Tel: +886 2 2516 0925, Fax: +886 2 2509 9756

**UNITED KINGDOM Phadia Ltd.**

Media House, Presley Way, Crownhill, Milton Keynes, MK8 0ES  
Tel: +44 1908 769 110, Fax: +44 1908 555 561












**USA Phadia US Inc.**

4169 Commercial Avenue, Portage, Michigan 49002  
Tel: +1 800 346 4364, Fax: +1 269 492 7541

**OTHER COUNTRIES Phadia AB**

Distributor Sales  
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**Phadia AB, P.O. Box 6460  
SE-751 37 Uppsala, Sweden  
Tel: +46 18 16 50 00  
Fax: +46 18 14 03 58**

