ImmunoCAP® Tryptase

Fluoroenzymeimmunoassay
Directions for Use 52-5467-EN/02

INTENDED USE

ImmunoCAP Tryptase is an in vitro test system for the quantitative measurement of tryptase in human serum or plasma. It is intended for in vitro diagnostic use in conjunction with other clinical findings, and is to be used in clinical laboratories. ImmunoCAP Tryptase is to be used with the instruments Phadia 100, Phadia 250 and Phadia 1000.

SUMMARY AND EXPLANATION OF THE TEST

Tryptase is the most abundant protein in mast cells. During IgE mediated allergic reactions mast cells are activated and release inflammatory mediators including tryptase (1,2,3). ImmunoCAP Tryptase measures the total tryptase levels including all forms of α-tryptase and ß-tryptase.

The baseline level of tryptase in the circulation reflects the number of mast cells.

Elevated basal levels of serum tryptase and/or an underlying mastocytosis may be risk factors particularly in patients with history of severe reactions. This should for example be taken into consideration in venom immunotherapy (8-10).

Mature ß-tryptase is transiently elevated in most cases of systemic anaphylactic reactions. The peak level is usually reached 15-120 minutes after onset of the reaction and tryptase levels then decline slowly within the next 3-6 hours. The return to baseline levels can generally be verified approximately 24 hours after the reaction (1,7).

Elevated tryptase levels in post-mortem samples may indicate a fatal anaphylactic reaction as a cause of death (11,12).

PRINCIPLE OF THE PROCEDURE

Anti-tryptase, covalently coupled to ImmunoCAP, reacts with the tryptase in the patient sample. After washing, enzyme labeled antibodies against tryptase are added to form a complex. Following incubation, unbound enzyme-anti-tryptase is washed away and the bound complex is then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate is measured. The higher the response value, the more tryptase is present in the sample. To evaluate the test results, the responses for the patient samples are transformed to concentrations with the use of a calibration curve.

REAGENTS AND MATERIAL

Reagents are packaged as described below, each purchased separately. The two digit suffix (-xx) on the article number may vary between countries. All kits are not available in all countries.

The expiration date and storage temperature are stated on the labels. Do not use reagents beyond their expiration dates.

Note: It is not recommended to pool any reagents.

Keep the ImmunoCAP carrier closed to avoid evaporation of buffer.

Reagents for Phadia 100

- **ImmunoCAP Tryptase** (Art No 10-9525-01: for 48 determinations)
  - Tryptase Conjugate (1 vial)
  - Tryptase Curve Control 1 (CC-1) (4 single dose vials)
- **ImmunoCAP Tryptase Calibrators** (Cal-xx) (Art No 10-9526-01: for 1 calibration curve)
- **ImmunoCAP Tryptase Curve Control** (CC-1) (Art No 10-9527-01: for 6 additional assay runs)
- **ImmunoCAP Tryptase Anti-Tryptase** (a-Tryp) (Art No 14-4518-01: carrier of 16 ImmunoCAP)
- **Development Solution** (Art No 10-9478-01: for 600 determinations)
- **Stop Solution** (Art No 10-9479-01: for 600 determinations)
- **Washing Solution** (Art No 10-9422-01: 6 x 1 l)
  - Washing Solution Additive, 6 x 17.2 ml
  - Washing Solution Concentrate, 6 x 80 ml
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Tryptase Control** (Art No 10-9370-01: 6 vials)

Reagents for Phadia 250

- **ImmunoCAP Tryptase Conjugate 50** (Art No 10-9522-01: for 2 x 50 determinations)
- **ImmunoCAP Tryptase Calibrator Strip** (Art No 10-9523-01: for 1 calibration curve)
- **ImmunoCAP Tryptase Curve Control Strip** (CC-1) (Art No 10-9524-01: for 6 x 1 curve controls)
- **ImmunoCAP Tryptase Anti-Tryptase** (a-Tryp) (Art No 14-4518-01: carrier of 16 ImmunoCAP)
- **Development Solution** (Art No 10-9441-01: for 6 x 200 determinations; Art No 10-9440-01: for 6 x 315 determinations)
- **Stop Solution** (Art No 10-9442-01: for 6 x 185 determinations)
- **Washing Solution** (Art No 10-9422-01: 6 x 1 l)
  - Washing Solution Additive, 6 x 17.2 ml
  - Washing Solution Concentrate, 6 x 80 ml
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Tryptase Control** (Art No 10-9370-01: 6 vials)

Reagents for Phadia 1000

- **ImmunoCAP Tryptase Conjugate 50** (Art No 10-9522-01: for 2 x 50 determinations)
- **ImmunoCAP Tryptase Calibrator Strip** (Art No 10-9523-01: for 1 calibration curve)
- **ImmunoCAP Tryptase Curve Control Strip** (CC-1) (Art No 10-9524-01: for 6 x 1 curve controls)
- **ImmunoCAP Tryptase Anti-Tryptase** (a-Tryp) (Art No 14-4518-01: carrier of 16 ImmunoCAP)
- **Development Solution** (Art No 10-9439-01: for 6 x 1200 determinations; Art No 10-9314-01: for 6 x 2000 determinations)
- **Stop Solution** (Art No 34-2271-51: for 1200 determinations)
- **Washing Solution** (Art No 10-9202-01: 2 x 5 l)
  - Washing Solution Additive, 2 x 86 ml
  - Washing Solution Concentrate, 2 x 400 ml
- **ImmunoCAP Tryptase Control** (Art No 10-9370-01: 6 vials)

Details of reagents

ImmunoCAP Tryptase Conjugate	
ß-Galactosidase-anti-tryptase Approximately 1.6 µg/ml (mouse monoclonal antibodies) Sodium azide 0.06%	Ready for use. Store at 2 – 8 °C until expiration date. **Do not freeze!**

ImmunoCAP Tryptase Calibrators/Calibrator Strip	
(human recombinant tryptase in buffer) Conc. 1; 5; 12.5; 50 and 200 µg/l Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Tryptase Curve Control/Curve Control Strip	
(human recombinant tryptase in buffer) Sodium azide 0.05%	Ready for use. Store at 2 – 8 °C until expiration date.

ImmunoCAP Tryptase Anti-Tryptase	
(mouse monoclonal antibodies) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

Development Solution	
4-Methylumbelliferyl-ß-D-galactoside 0.01% Preservative* <0.0010%	Ready for use. Store at 2 – 8 °C until expiration date. **Do not freeze!**

Stop Solution	
Sodium carbonate 4%	Ready for use. Store at 2 – 32 °C until expiration date.

Washing Solution
For information, see separate Directions for Use for Washing Solution.

ImmunoCAP Tryptase Control
ImmunoCAP Tryptase Control is prepared from selected pooled human sera. It is lyophilized to ensure maximum stability. **Reconstitution of ImmunoCAP Tryptase Control** Reconstitute the content of a vial by adding exactly 500 µl purified water (18, 19) or clinical laboratory reagent water (CLRW, 20). Let the vial stand for one minute, then mix gently until the content is completely dissolved. **Shelf-life and storage** Lyophilized serum: Store at 2-8 °C until expiration date. Reconstituted serum: Store at 2 – 8 °C for 1 week or -20 ° C for 4 weeks. (Repeated freezing and thawing should be avoided.)

ImmunoCAP IgE/ECP/Tryptase Sample Diluent	
(buffer solution with Bovine Serum Albumin) Preservative* <0.003%	Ready for use. Store at 2 – 8 °C until expiration date.

***Preservative:** Mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1).

Additional material

Additional products available from Phadia AB:

- ImmunoCAP IgE/ECP/Tryptase Sample Diluent (10-9256-01/10-9360-01)
- Maintenance Solution Kit (10-9476-01)

Materials required but not provided by Phadia AB:

- Measuring cylinder 1000 ml
- Purified water (18,19) or Clinical Laboratory Reagent Water (CLRW, 20)

⚠ Precautions

- For in vitro diagnostic use. Not for internal or external use in humans or animals.
- Some reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found to be negative. 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.
- Reagents containing >0.0015% mixture of 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2*H*-isothiazol-3-one [EC no. 220-239-6] (3:1) may cause sensitization by skin contact. Avoid contact with skin. Wear suitable gloves. For more information see Safety Data Sheet.

INSTRUMENTS

Phadia 100 processes all steps of the assay and prints results automatically after the assay is completed[(a)]. Phadia 250 and Phadia 1000 are continuous random access systems that perform all steps of the assay[(a)].

On board stability

Phadia 100 has no provisions for on board reagent storage. Information on reagent storage for other Phadia instruments is shown below.

	Phadia 250	**Phadia 1000**
Calibrator/ Curve Control Strip	28 days[B].	
Conjugate	4 days open[A]. Recap bottles every night.	7 days open[A].
Development Solution	5 days open[B]. Recap bottles every night.	14 days open[A].
Stop Solution	Until expiration date[B]. Recap bottles every night.	Until expiration date[B].
Washing Solution (prepared solution)	7 days[B]. Discard every seventh day and perform weekly maintenance according to respective instrument user manual.	
Washing Solution Concentrate	N/A	

	Phadia 250	Phadia 1000
Washing Solution Additive	N/A	
Sample Diluent	7 days[B]. Recap bottles every night.	N/A
ImmunoCAP Carrier	Until expiration date[A].	

[A]Refrigerated (2-8 °C)

[B]Room temperature (18-32 °C)

SPECIMEN COLLECTION AND PREPARATION

Specimen collection

Serum and plasma (EDTA or heparin) samples from venous blood can be used. Collect blood samples and prepare serum or plasma according to standard procedures. Keep specimens at room temperature (RT) for shipping purposes only, up to 2 days. Store at 2 °C to 8 °C for up to one week, or else at –20 °C. Avoid repeated freezing and thawing (16).

It is preferable that samples be taken no earlier than 15 minutes from onset / up to 3 hours after the onset of the suspected incident causing mast cell activation (5,6). The time between the reaction and sample collection should be noted. To confirm the return to baseline levels an additional blood sample should be collected after 24 - 48 hours, time depending on the magnitude of the activation. At the suspicion of elevated basal levels or an underlying mastocytosis additional sample(s) should be taken 1-2 week(s) later.

Post mortem samples should be taken within 48 hours from time of death.

Preparation of samples

Sample dilution is usually not required.

For determination of values higher than 200 µg/l, dilute the samples with ImmunoCAP IgE/ECP/Tryptase Sample Diluent.

Handling of control specimen

It is recommended to remove and recap the control vials from the instrument as soon as the pipetting of the samples is finished and the sample incubation is started. It is also recommended to gently stir the vial before use. Avoid repeated freezing and thawing.

ImmunoCAP Tryptase Control should be treated in the same way as a patient sample in the procedure.

PROCEDURES

Procedural steps[(a)]

For procedural steps, see **Notes** a.

Parameters of the procedure

Patient samples are run in single determinations.

Volumes per determination:

Sample	40 µl
Conjugate	50 µl
Development Solution	50 µl
Stop Solution	600 µl

Incubations are performed at 37 °C by Phadia instruments.

Phadia 100: Total time for one assay is 2.5 hours.

Phadia 250 and Phadia 1000: The process time is 1 hour and 45 minutes from entering the first sample until the result is available.

Calibration[(a)]

The ImmunoCAP Tryptase Calibrators or ImmunoCAP Tryptase Calibrator Strip are run in duplicates to obtain a full calibration curve. The curve can be stored. The software for Phadia instruments have built-in acceptance limits for the calibration curve and the curve controls. Use one curve control, CC-1, in duplicate determinations to evaluate subsequent assays against the stored curve.

Calibrator range: 1-200 µg/l.

Reference material: ImmunoCAP Tryptase Calibrators are calibrated against an in-house tryptase reference which traces back to tryptase purified according to Schwartz et al (13).

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 Specimen

Good laboratory practice requires that quality control specimen should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptable ranges.

Controls available from Phadia AB for day to day quality control:

- ImmunoCAP Tryptase Control (10-9370-01)

Intended use

ImmunoCAP Tryptase Control is used for monitoring ImmunoCAP Tryptase measurements performance in Phadia instruments.

Expected values for ImmunoCAP Tryptase Control

As with all immunoassays the results are affected by the testing procedures and equipment used by different laboratories. It is therefore recommended that each laboratory establishes its own target value for each actual lot of control together with criteria of acceptance (recommended range ± 30 %).

This established target value is expected to fall within the range for the actual lot stated on the vial label.

The range for each specific lot is calculated as a mean ± 3 SD using the expected long term variation. The mean value for each specific lot has been determined from 6 consecutive control assays, each in 6 replicates using ImmunoCAP Tryptase.

RESULTS

For Phadia instruments using Phadia Information Data Manager Software, all calculations are automatically performed[(a)]. Phadia 100 is programmed to automatically calculate all results[(a)]. It can also be used with Phadia Information Data Manager Software.

LIMITATIONS OF THE PROCEDURE

A definitive clinical diagnosis should only be made by the physician after all clinical and laboratory findings have been evaluated. It should not be based on the results of any single diagnostic method.

- Systemic anaphylactic reactions without elevation of tryptase might be caused by other non-mast cell pathways.
- In general, elevated tryptase levels are more commonly seen after parenteral than oral introduction of agents causing systemic anaphylactic reactions.
- Moderately elevated tryptase levels have been observed in post-mortem samples by other death causes than anaphylactic reactions.
- Heterophilic antibodies, especially human-anti mouse antibodies (HAMA), in human serum/plasma might react with the mouse immunoglobulins used as capturing antibodies on the solid phase and as detection antibodies in the conjugate in ImmunoCAP Tryptase (14,15). The presence of heterophilic antibodies is uncommon but can cause false results, mostly falsely elevated levels. The composition of the anti-tryptase conjugate is designed in order to minimize this kind of interference.
  Still uncommon, the risk for interference is increased in certain patient groups e.g. patients having rheumatoid factor (RF) or patients receiving preparations containing mouse monoclonal antibodies (including chimeric/humanized) for diagnostic and/or therapeutic use. Another risk group is patients regularly exposed to animals and/or animal products.

  One method to confirm the presence of heterophilic antibodies is by pre-treatment of samples using commercially available Heterophilic Blocking Tubes (HBT). For use and limitations of HBT, see manufacturer's instructions.

EXPECTED VALUES[(b)]

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

A study[(b)] with 124 self-reported healthy individuals (56 males and 68 females) was performed with Phadia 250. The age range was 3-67 years for males and 4-63 years for females.

The following ImmunoCAP Tryptase results were obtained:

Geometric mean	3.4 µg/l
95 upper percentile	11.0 µg/l

In patients with systemic mastocytosis levels of tryptase are, in general, persistently elevated above 20 µg/l (4).

Baseline tryptase levels in the range of approximately 10-20 µg/l reflect an increased mast cell burden indicating an increased risk in patients with history of severe anaphylactic reaction (1).

In severe cases the triggering agent causing a transiently elevation of tryptase should be identified.

PERFORMANCE CHARACTERISTICS

Instrument comparison

Comparison studies[(b)] have been performed with different combinations of Phadia instruments, including at least 50 patient samples. The results obtained show good concordance between instruments.

Precision[(b)]

The following pooled coefficients of variation have been obtained with Phadia 250 and 4-10 samples/level. Each sample has been assayed in 4 replicates on 7 different occasions on each of 3 different instruments, using the same lot of reagents and stored calibration curves. The values are also representative for Phadia 100 and Phadia 1000.

Sample level (µg/l)	Coefficients of variation (%)	
	Within assay	Between assay
1-20	3	5
20-100	3	6
100-200	4	7

Analytical sensitivity[(b)]

The limit of detection (17) is 1.0 µg/l.

Analytical specificity[(b)]

No interference of Heparin, Rheumatoid factor, hemolysed, lipemic or icteric samples has been observed.

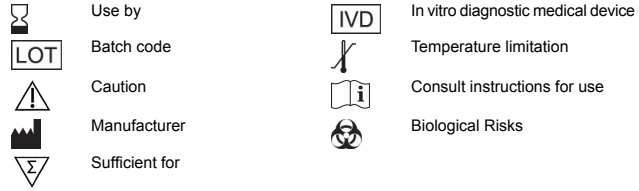
Recovery[(b)]

Mean recovery is 100±7.5%.

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 and its authorized distributors, in such event, shall not be liable for damages indirect or consequential.

SYMBOLS



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Notes

[a]For more information, see Phadia 100, Phadia 250 and/or Phadia 1000 User Manual.

[b]Studies performed at Phadia AB, Uppsala, Sweden.

Patents/Trademarks

Addresses

AUSTRIA Phadia Austria GmbH
Donau-City-Straße 1
AT-1220 Vienna
Tel: +43-1 270 2020 Fax: +43-1 270 202020
BELGIUM Phadia NV/SA
Pontbeekstraat 2
BE-1702 GROOT-BIJGAARDEN
Tel: +32-2 749 55 15 Fax: +32-2 749 55 23
BRAZIL Phadia Diagnósticos Ltda.
Rua Luigi Galvani, 70-10° andar - conj. 101
Cidade Monções - São Paulo – SP
Cep: 04575-020
Tel: +55-11 3345 5050 Fax: +55-11 3345 5060
CHINA Thermo Fisher Scientific (China) Co., Ltd.
Building 6-7
No. 27 Xin Jin Qiao Road
Shanghai 201102
Tel. +86 800 810 5118 Fax. +86 400 650 5118
CZECH REPUBLIC Phadia s.r.o.
Drahobejlova 1019/27
19000 PRAHA 9
Tel: +420 220 518 743 Fax: +420 220 518 743
DENMARK Phadia ApS
Gydevang 33
DK-3450 ALLERØD
Tel: +45-70 23 33 06 Fax: +45-70 23 33 07
FINLAND Phadia Oy
Ratastie 2
P.O. Box 100
FIN-01621 VANTAA
Tel: +358-9 3291 0110 Fax: +358-9 3291 0531
FRANCE Phadia S.A.S.
BP 610
FR-78056 ST QUENTIN-EN-YVELINES CEDEX
Tel: +33-1 61 37 34 30 Fax: +33-1 30 64 62 37
GERMANY Phadia GmbH
Postfach 1050 DE-790 10 FREIBURG
Tel: +49-761 47 805-0 Fax: +49-761 47805-338
HONG KONG
Thermo Fisher Scientific (Hong Kong) Limited
Unit 11-15, 9/F Tower 1
Grand Central Plaza
138 Shatin Rural Committee Road, Shatin
New Territories, Hong Kong
Tel: +852 2885 4613 Fax: +852 2567 4447
INDIA Phadia India Pvt. Ltd
Unit No.07, 10 & 11, ground floor,
Splendor forum, plot no 03
Jasola, District Centre,
NEW DEHLI-110025
Tel: +91 11 461 075 55 / 56 Fax: +91 11 461 075 57
IRELAND Phadia Ltd. (Irish Branch)
27 Oakhill
Moate
Co. Westmeath
Tel: +44 1800 625 167 Fax: +44 1800 625 168
ITALY Phadia S.r.l.
Via Libero Temolo, 4
IT-201 26 MILANO
Tel: +39-0264 163 411 Fax: +39-0264 163 415
JAPAN Phadia K.K.
NBF Ueno Bldg 9th Floor,
4-24-11, Higashi -Ueno Taito-ku,
Tokyo 110-0015, Japan
Tel: +81-3-5826-1660 Fax: +81-3-5826-1692
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 Thermo Fisher Scientific
Phadia AS
Postboks 4756, Nydalen
NO-0421 OSLO
Tel: +47-21 67 32 80 Fax: +47-21 67 32 81
PORTUGAL Phadia Sociedade Unipessoal Lda
Lagoas Park - Edifício n°11 - Piso 0
PT-2740-270 PORTO SALVO
Tel: +351-214 23 53 50 Fax: +351-214 21 60 36
SOUTH AFRICA Laboratory Specialities (PTY)
A Phadia Company
P.O. Box 1259
Ferndale 2160
Tel: +27 11 792 6790 Fax: +27 11 793 1064
SPAIN Phadia Spain SL.
Avda. Alcalde Barnils nº 70 Planta 2
Edificio Onada
08174 Sant Cugat del Vallés, Barcelona
Tel: +34-935 765 800 Fax: +34-935 765 820
SWEDEN Phadia AB,
Marknadsbolag Sverige
P O Box 6460 SE-751 37 UPPSALA
Tel: +46-18 16 60 60 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 Thermo Fisher Scientific Taiwan Co., Ltd.
6F-1, No. 85, Jhouzih St., NeiHu District
Taipei City 11493
Tel. +886 2 8751 6655 Fax. +886 2 8751 5353
UNITED KINGDOM Phadia Ltd
16 Shenley Pavilions, Chalkdell Drive
Shenley Wood, Milton Keynes, MK5 6LB
Tel: +44-1908 76 91 10 Fax: +44-844 324 94 95
USA Phadia US Inc.
4169 Commercial Avenue
Portage, Michigan 49002
Tel: +1 800-346-4364 (Toll Free) Fax: +1 269 492-7541
OTHER COUNTRIES Phadia AB,
Distributor Sales
P O Box 6460, SE-751 37 UPPSALA
Tel: +46 18 16 50 00 Fax: +46 18 16 63 65

Phadia AB,
Rapsgatan 7P, P. O. Box 6460, 751 37 Uppsala, Sweden
Tel: +46 18 16 50 00 Fax: +46 18 14 03 58

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