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Manual

IDK® Tryptase ELISA

For the in vitro determination of tryptase in EDTA-plasma

Valid from 2024-01-30

REF **K 6814**

Σ 96

+8°C
+2°C

IVD

CE

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1. INTENDED USE

The assay described here is suitable for the determination of tryptase from **EDTA plasma**. For *in vitro* diagnostic use only.

2. INTRODUCTION

Tryptase is a collective term for a family of trypsin-like serine proteases. They are mainly produced and stored in mast cells to be released into the bloodstream upon activation [1, 2]. Tryptases are vasoactive, proinflammatory and have a chemotactic effect on leukocytes. In this context, they play a special role in inflammatory processes and type I allergies. Elevated tryptase concentrations in plasma are also found in myeloid diseases; in contrast to plasma with a lymphoid neoplasm, who tend to have normal tryptase concentrations [4, 5].

Indications

- myeloid diseases and neoplasms, incl. Mastocytosis
- anaphylactic reactions (drugs, insect venom)
- drug hypersensitivity (e.g. anesthetics or contrast media)

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
K 6814	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
K 6814	AB	Detection antibody concentrate , biotinylated	1 x 200 µl
K 6814	CONJ	Conjugate concentrate, peroxidase-labelled	1 x 200 µl
K 6814	STDKONZ	Standard concentrate, lyophilised	4 x 1 vial
K 6814	CTRL1	Control 1, lyophilised (see specification for range)	4 x 1 vial
K 6814	CTRL2	Control 2, lyophilised (see specification for range)	4 x 1 vial
K 6814	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml

Cat. No.	Label	Kit components	Quantity
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1 000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge
- Vortex
- Standard laboratory glass or plastic vials, cups, absorbent paper etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- The **lyophilised standard concentrate (STDKONZ)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standard concentrate and controls** (reconstituted STDKONZ and CTRL) **are not stable and cannot be stored.**
- The preparation of the **standard curve** (volumes and concentrations) is described in the product specification.
- **Preparation of the detection antibody and the conjugate:** Before use, the **detection antibody concentrate (AB)** and the **conjugate concentrate (CONJ)** have to be diluted **1:101** in **dilution buffer** (100 µl AB + 10 ml Waschpuffer und 100 µl CONJ + 10 ml Waschpuffer). The AB and the CONJ are stable at **2–8 °C** until the expiry date stated on the label. **Detection antibody** (1:101 diluted AB) and **conjugate** (1:101 diluted CONJ) **are not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

EDTA plasma samples

The sample material is stored at -20 °C until use. **Important:** once thawed, analyse samples immediately if possible, avoid repeated freeze-thaw cycles.

Sample preparation

We recommend to **centrifuge thawed** EDTA plasma in 1.5 ml reaction tubes at 10 000 *g* for **5 min** before use in the assay.

Sample dilution

EDTA-Plasma samples must be diluted **1:5** before performing the assay,

e.g. **50 µl** sample + **200 µl** sample dilution buffer (SAMPLEBUF), mix well.

100 µl of the dilution are used in the test.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of tryptase.

In a first incubation step, tryptase in the sample is bound to tryptase antibodies, which are immobilised on the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out.

In a second incubation step, a biotinylated polyclonal anti-tryptase antibody is added into each microtiter well. After a further washing step, the streptavidin peroxidase conjugate is added and a "sandwich"of

1st antibody – tryptase - biotinylated antibody – streptavidin peroxidase conjugate

is formed.

Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. An acidic stop solution is then added to terminate the reaction. The colour changes from blue to yellow. The intensity of the yellow colour ist directly proportional to the tryptase content in the sample.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. Tryptase, present in the samples, is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered in the aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at **37 °C** on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl detection antibody** (diluted AB) into each well.
6.	Cover the strips and incubate for **1 hour** at **37 °C** on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl conjugate** (diluted CONJ) into each well.
9.	Cover the strips and incubate for **1 hour** at **37 °C** on a **horizontal shaker***.
10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
13.	Add **100 µl stop solution** (STOP) into each well and mix well.
14.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

EDTA plasma samples

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

Analytical sensitivity × *sample dilution factor to be used*

Analytical Sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB	0.391 ng/ml
Limit of detection, LoD	0.749 ng/ml

The evaluation was performed according to the CLSI guideline EP17-A2.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

We recommend each laboratory to establish its own reference range.

11. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide or ProClin are hazardous to health and the environment. Substrates for enzymatic colour reactions may also cause skin and/or respiratory irritation. Any contact with the substances must be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: get medical Advice/attention.

- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

12. DISPOSAL

- Liquid test components, pipets tips, tubes etc. are to be treated as ordinary laboratory waste, unless otherwise stated. The solutions should be discarded in a proper container after testing following local regulations.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. Other potentially infectious materials (e.g. sample collection container) must be disposed in accordance with official regulations.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Vitte J. Human mast cell tryptase in biology and medicine. Mol Immunol; 2015
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5. Sperr WR, Stehberger B, Wimazal F, Baghestanian M, Schwartz LB, Kundi M, Semper H, Jordan JH, Chott A, Drach J, Jäger U, Geissler K, Greschniok A, Horny HP, Lechner K, Valent P. Serum tryptase measurements in patients with myelodysplastic syndromes. Leuk Lymphoma 2002;43:1097-105.

Used symbols:

 Temperature limitation

REF Catalogue number

IVD In Vitro Diagnostic Medical Device

→REF To be used with

 Manufacturer

 Contains sufficient for <n> tests

LOT Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet

 Irritant