Package Insert

HUMAN

ELISA sphingotest® penKid®

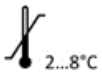
Enzyme-linked immunosorbent assay for the quantification of Proenkephalin A 119-159 in human EDTA plasma.

For Research Use Only. Not for Diagnostic Procedures.

English

REF 080-05000

RUO



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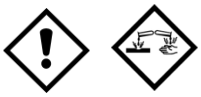

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Orders

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Intended use

The ELISA sphingotest® penKid® is a non-automated one-step enzyme-linked immuno-sorbent assay (ELISA) for the quantitative measurement of **Proenkephalin A 119-159** in human EDTA plasma. The product is for research use only. It is not intended for diagnostic purposes.

Background information

Proenkephalin A 119-159 (penKid) is a stable mid-regional fragment of Proenkephalin A and has been established as a surrogate plasma marker for the unstable enkephalin peptides that are derived from the same precursor (1-3). Enkephalins are endogenous pentapeptides that act via opioid receptors. Enkephalin and its receptors are highly abundant in the kidney (2, 4).

Test principle

The ELISA sphingotest® penKid® is a non-automated ELISA for the quantitative measurement of Proenkephalin A 119-159 in human EDTA plasma.

Three antigen-specific monoclonal antibodies are used that bind penKid (antigen) at three different epitopes. One of the antibodies is labelled with horseradish peroxidase (HRP) (tracer antibody), while the other two antibodies are coated to the microplate wells.

During the 2-hour incubation period, the antibodies react with the penKid molecules in the sample forming a sandwich complex (figure 1). In this way, the tracer antibody is indirectly bound to the surface of the microplate. Afterwards, any unbound material is completely removed by careful washing.

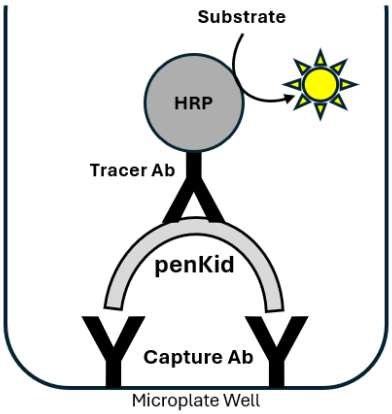

Figure 1: Test principle with representation of the sandwich complex.

A substrate solution is added to the washed wells containing tetramethylbenzidine (TMB) that is catalysed by the bound HRP to generate a blue coloured product. The enzymatic reaction is chemically stopped with a colour change to yellow, and the colour intensity is read at 450 nm (reference wavelength 570 nm) using a suitable microplate reader.

The absorbance signal is directly proportional to the penKid concentration of the respective plasma sample. Parallel measurement of the included calibrators, with lot-specific assigned concentrations of synthetic human penKid, enables the preparation of a calibration curve, through which the unknown penKid concentration in the sample can be deduced.

Reagents and materials provided

Material	Description and Reconstitution
CALIBRATOR (PELCA) 1 vial, lyophilized	Lyophilized, 1 vial is provided that has to be reconstituted with 500 µL ZERO MATRIX. It is used to generate the calibrator levels.
ZERO MATRIX (PELZM) 1 bottle, 4 mL, liquid GHS07  Warning	Ready-to-use ZERO MATRIX for reconstitution of CALIBRATOR and generation of calibrator levels. Hazard statement*: H317 Precautionary statements*: P261, P280, P302+P352, P333+P313, P501

MICROPLATE (PELMP) 1 plate with 96 cavities	Ready-to-use microplate coated with anti-human-penKid antibodies (mouse monoclonal)
TRACER (PELTR) 1 bottle, lyophilized	Lyophilized HRP-labelled anti-human-penKid antibody in reaction buffer. To be reconstituted in 18 mL BUFFER.
BUFFER (PELBU) 1 bottle, 18 mL, liquid GHS07  Warning	Ready-to-use buffer solution for reconstitution of TRACER. Hazard statement*: H317 Precautionary statements*: P261, P280, P302+P352, P333+P313, P501,
WASH CONCENTRATE (PELWA) 1 bottle, 22 mL, liquid	Concentrated wash solution. To be diluted to 1,100 µL with pure water.
SUBSTRATE (PELSU) 1 bottle, 11 mL, liquid	Substrate solution containing TMB for enzymatic reaction of HRP.
STOP SOLUTION (PELST) 1 bottle, 6 mL, liquid GHS05  Warning	Stop solution, to chemically stop HRP enzymatic reaction. Hazard statement*: H290, H315, H319 Precautionary statements*: P280, P302+P352, P305+P351+P338, P337+P313
2 Adhesive foil	Black colored adhesive foil for light protected assay incubation. Used to cover sampled microplate during incubation.
1 Package Insert	Print version of the package insert
1 Quality Report (QR)	Print version of the quality report

Reagents and materials required but not provided

- Microlitre pipettes covering a range of 50 – 500 µL with replaceable plastic tip.
- Multichannel pipette (150 µL) with replaceable plastic tip.
- Polypropylene tubes (≥500 µL).
- Vortex mixer.
- Reagent reservoirs with capacity ≥18 mL (for pipetting tracer, substrate and stop solutions using a multichannel pipette).
- Laboratory water grade 2 (acc. ISO 3696:1987).
- Bottle with lid or screw cap (1,100 mL) to fit the ready-to-use wash solution

Special material required but not provided

- Automated microplate washing device (e.g. Wellwash, Thermo Scientific).
- Microplate reader capable of reading absorbances at 450 nm and 570 nm (e.g. SpectraMax ID5, Molecular Devices LLC).

Key performance characteristics of device(s) and equipment to combine

The microplate reader used in conjunction with the ELISA sphingotest® penKid® must have the minimum specifications as given in the table below:

Parameter	**Specification**
MTP type	96-well, flat-bottom
MTP dimension (LxWxH)	128.2 x 86.0 x 14.77 mm
Detection principle	Absorbance
Wavelengths	450 nm, 570 nm
Wavelengths accuracy	± 2.0 nm
Photometric range	Up to 4.0 OD
Photometric resolution	0.001 OD

Storage and handling of reagents

All reagents must be stored at 2°C to 8°C in the kit packaging until usage. The expiry date specified on the kit packaging and reagents must be observed under all circumstances.

The ready-to-use washing solution can be used for up to four weeks when stored at room temperature. Microbial-contaminated washing solution should be discarded. Contamination can be identified through turbidity or a pH < 6.

Warnings and precautions

- For research use only. Not for diagnostic procedures.
- The product must only be used by professional users in a laboratory setting.

- Read the instructions for use carefully before performing the test. Test performance can be affected if reagents are improperly prepared or stored under conditions other than those indicated.
- Do not freeze any parts of the test kit.
- Do not use kit components after the expiry date has passed.
- Store the test kit at 2...8°C.
- Do not use damaged kits.
- Wear protective clothing such as laboratory coats, eye/face protection and disposable gloves whenever kit components and human specimens are handled.
- Wear nitrile butadiene rubber (NBR) gloves with permeation level 6 when handling the stop solution.
- Components of this kit contain substances of animal origin.
- Samples should be considered potentially infectious and treated accordingly. At present, no method can guarantee the complete absence of infectious components. All materials should be disposed of as potentially infectious and according to local regulations.
- The microplate should remain in the sealed pouch until use.
- Bring the test kit and reagent to room temperature before use.
- Use a new pipette tip for each well. Use only unused, disposable material.
- Do not use more than the required amount of liquids.
- During the incubation periods, proper sealing of the microplate using the adhesive foil supplied will protect it from light and prevent sample drying and ensure reproducibility of the results.
- Carefully close all containers after completion of the test.
- Prepared or used reagents and chemicals must be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

Hazards and Precautions	**Component**
May be corrosive to metals (H290)	STOP SOLUTION GHS05  Warning
Causes skin irritation (H315)	
Cause serious eye irritation (H319)	
If on skin, wash with plenty of water (P302+P352)	
If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing (P305+PS351+P338)	
If eye irritation persists: Get medical advice/attention (P337+P313)	
May cause allergic skin reaction (H317)	ZERO MATRIX, BUFFER GHS07  Warning
Avoid breathing dust/fume/gas/ mist/vapours/spray (P261)	
If on skin, wash with soap and water (P302+P352)	
If skin irritation or rash occurs, get medical advice (P333+P313)	

Sample collection and handling

- Collect whole blood in a suitable EDTA plasma collection tube.
- Separate the plasma by spinning the collection tube following the manufacturer's instructions.
- Carefully transfer the plasma into a fresh vial.
- If only heparin plasma is available, a matrix correction factor will need to be applied on results (see chapter "Calculation of results")
- Do not use plasma with citrate as coagulant.
- Do not use lipemic, haemolytic or contaminated plasma.

Sample preparation

- When samples from refrigerated or frozen conditions are used, allow them to incubate to ambient temperature for at least one hour.
- The native sample material is used without dilution.
- Bring all kit components to ambient temperature.
- Prepare the reagents as described in the chapter "Reagent preparation".
- All liquid components – including the samples – should be mixed before use (avoid foam formation).
- If applicable, prepare an assignment plan for the microtiter plate wells.

Preparation of the assay

Remove the test kit from its storage condition and allow it to reach ambient temperature.

Reagent preparation

1. Reconstitute CALIBRATOR by adding 500 µL of ZERO MATRIX. Gently mix for 10 minutes and ensure that all lyophilised material is completely resolved. The reconstituted calibrator serves as stock solution for generation of the calibrator levels.

2. Generate in total six (6) calibrator levels (CAL6 to CAL1) by serial dilution (dilution factor 2.5) using the CALIBARTOR and ZERO MATRIX. Pipette 300 µL ZERO MATRIX to six (6) new polypropylene tubes. Add 200 µL of CALIBRATOR to the first vial resulting in CAL6. Use CAL6 to generate CAL5 by pipetting 200 µL. Repeat this procedure until CAL1 is generated (figure 2). Mix the vials carefully between pipetting steps.

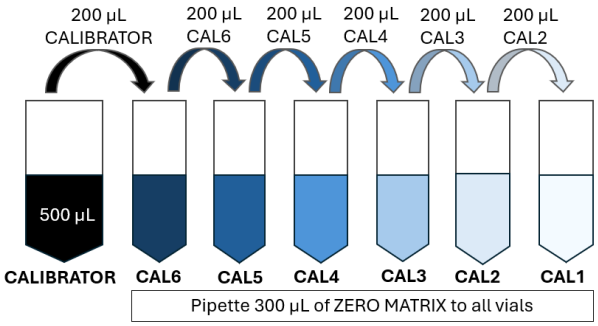

Figure 2: Schematic representation of serial calibrator dilution.

3. The remaining ZERO MATRIX serves as zero calibrator (CAL0). The seven ready to use calibrators (including CAL0) will have concentrations as given in the lot-specific quality report.
4. Reconstitute the TRACER by adding approx. half the volume (approx. 9 mL) of the BUFFER to the TRACER bottle. Retain the other half in the BUFFER bottle. Gently mix for 10 minutes and ensure that all lyophilised material is completely resolved. Transfer the solution to the BUFFER bottle to obtain the ready-to-use TRACER solution (18 mL).
5. Prepare the washing solution by diluting the WASH CONCENTRATE to 1,100 mL with laboratory water (grade 2).

Assay procedure

Pipette calibrators and samples according to the following scheme (figure 3). Calibrators and samples are measured in duplicates.

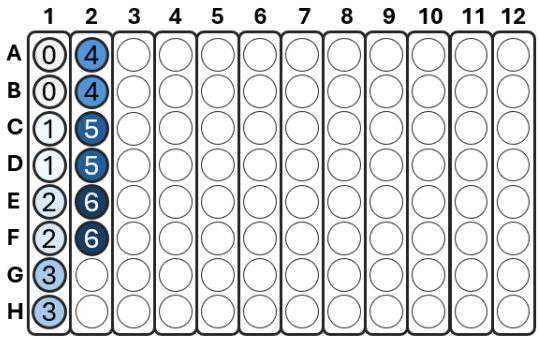

Figure 3: Recommended pipetting scheme. The six generated calibrator levels are shown with numbers and color code.

1. Pipette 50 µL of each calibrator level (CAL0-CAL6) in duplicates and 50 µL of up to 41 patient samples in duplicates.
2. Pipette 150 µL Tracer solution to each well using a multichannel pipette.
3. Cover the plate with the adhesive foil provided. Incubate the plate for 2 hours ± 15 min at ambient conditions (18-29°C) without agitation.
4. Aspirate the reaction mixture from all wells, the use of an automatic washing device is recommended.
5. Wash each well 4x with 350 µL washing solution; the use of an automatic washing device is recommended.
6. Pipette 100 µL of ready-to-use SUBSTRATE to each well using a multichannel pipette.
7. Cover the plate with the second adhesive foil provided. Incubate the plate for 20-30 minutes at ambient conditions (18-29°C) with 150 rpm agitation.
8. Pipette 50 µL of STOP SOLUTION to each well using a multichannel pipette. The colored wells will change from blue to yellow indicating that the reaction enzymatic HRP reaction was stopped.
9. Measure the absorbance for each well in a suitable calibrated microtiter plate reader at 450 nm wavelength. Use 570 nm as reference wavelength (for signal correction). The instrument shall automatically subtract the signal at 570 nm from the main signal at 450 nm. In case the device is not capable in measuring two wavelength the same time, measure both wavelengths separately after each other. Manually subtract the signal at 570 nm from that at 450 nm.

Calculation of results

The averaged corrected absorbance signals (450 nm – 570 nm) obtained for the calibrators are used for calibration. The signal for each calibrator (y-axis) and its respective target concentration (x-axis) is used to apply a four-parametric logistic fit to generate the calibration curve. The penKid concentrations (pmol/L) for all measured patient samples are deduced from this calibration curve using the averaged corrected absorbance signal from the respective duplicate sample measurement.

If heparin plasma is used instead of EDTA plasma, multiply the obtained sample concentrations by 1.32 to obtain the final concentration.

Calculation example

Example absorbances measured on a SpectraMax® ID5 (Molecular Devices LLC) are shown in the table below.

Calibrator level	Assigned penKid concentration (pmol/L)	Averaged corrected absorbance (450 nm – 570 nm)
CAL 0	0.0	0.011
CAL 1	9.8	0.044
CAL 2	24.5	0.094
CAL 3	61.2	0.236
CAL 4	153	0.612
CAL 5	383	1.509
CAL 6	957	3.114

The obtained calibration curve applying a four-parametric logistic curve is shown in figure 4 (for better visibility the axes are in log-scale):

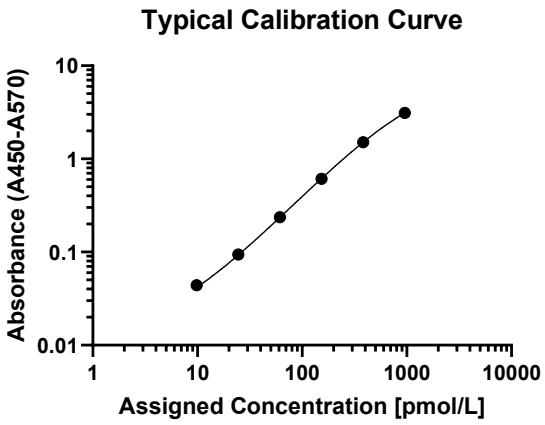

Figure 4: Exemplary calibration curve. CAL0 is included in the fit but not shown due to the logarithmic scale of both axes.

Technical performance characteristics

Traceability

The ELISA sphingotest® penKid® is calibrated by weighted synthetic Proenkephalin A 119-159. The sphingotest® penKid® chemiluminescence assay (CLIA) serves as the reference calibration for this product (3).

Accuracy of measurement

A method comparison between ELISA sphingotest® penKid® and the sphingotest® penKid® CLIA (3) using 120 EDTA plasma samples covering the measuring range estimated an average bias of -0.5%, a slope of 0.925 and a correlation (Spearman rs) of 0.980.

Measuring range and linearity

The measuring range is 10.2 to 1,000 pmol/L. The actual lot-specific calibration range is given in the quality report. A linearity study was conducted testing eleven concentrations in fivefold determination by mixing two EDTA samples spanning the measuring range.

Detection capability

The limit of detection was determined as lowest measurement result with a 95% probability of claiming the presence of penKid. The limit of quantification is defined as the lowest measurement result detectable with a coefficient of variation (CV) of 20% (intra-laboratory precision).

LoD	8.9 pmol/L
LoQ (20% Total CV)	10.2 pmol/L

Precision of measurement

The precision of the ELISA sphingotest® penKid® was determined in a single site study using three EDTA samples with each 80 determinations.

Sample	penKid [pmol/L]	Repeatability CV	Reproducibility CV
1	34.9	8.3%	10.5%
2	79.9	1.9%	6.7%
3	302.8	2.0%	4.9%

High dose hook effect (HDH)

The ELISA sphingotest® penKid® shows no observational signal loss due to high penKid concentrations up to 10,000 pmol/L, which is 10-times the upper measuring range of the assay.

Notice to the user

For getting the appropriate Safety Data Sheets according to EC directive 1907/2006 (REACH) or technical assistance and more information please contact our distribution partner or SphingoTec GmbH (see contact information on the kit label).

Literature

1. Ernst A, Kohrle J, Bergmann A. Proenkephalin A 119-159, a stable proenkephalin A precursor fragment identified in human circulation. Peptides. 2006;27(7):1835-40.
2. Beunders R, Struck J, Wu AH, Zarbock A, Di Somma S, Mehta RL, et al. Proenkephalin (PENK) as a novel biomarker for kidney function. J Appl Lab Med. 2017;DOI: 10.1373/jalm.2017.023598 400-12.
3. Donato LJ, Meeusen JW, Lieske JC, Bergmann D, Sparwasser A, Jaffe AS. Analytical performance of an immunoassay to measure proenkephalin. Clinical biochemistry. 2018;58:72-7.
4. Denning GM, Ackermann LW, Barna TJ, Armstrong JG, Stoll LL, Weintraub NL, et al. Proenkephalin expression and enkephalin release are widely observed in non-neuronal tissues. Peptides. 2008;29(1):83-92.

Symbols

Symbol	Application	Symbol	Application	Symbol	Application
	Consult instructions for use	REF	Article Number		Do not re-use
RUO	For research use only. Not for diagnostic procedures.		Contents sufficient for (number of) tests		Use by date
	Temperature limit	LOT	Batch code		Green dot according to German legislation
	Distributor	RCNS	Reconstitution		Manufacturer
LYOPH	Lyophilized	HUMAN	Assay reactivity: Human		GHS05
	GHS07				

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Document Revision History

Revision No.	Date	Changes
01	March 2026	Initial release