Mercodia

HNL Homodimer ELISA

Directions for Use

10-1380-01
Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

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Explanation of symbols used on labels

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.

Intended Use

HNL Homodimer ELISA is intended to be used for the in vitro quantitative measurement of homodimeric Human Neutrophil Lipocalin (HNL) in human plasma.

Summary and explanation of the test

Human Neutrophil Lipocalin (HNL)[1], also known as Neutrophil Gelatinase-Associated Lipocalin (NGAL)[2], is a glycoprotein that exists in multiple forms, including a 45-kDa disulfide-linked homodimer. This homodimeric form of HNL is a significant component of the secondary granules in human neutrophil granulocytes and is released into the extracellular environment upon activation of the cells.[1,3]

Studies[4,5] have demonstrated that plasma levels of the HNL homodimer can effectively monitor the success of antibiotic treatment in sepsis patients, providing faster and more reliable responses compared to traditional biomarkers. This positions the HNL homodimer as a promising future tool, with the potential to significantly enhance patient outcomes by enabling timely and targeted therapeutic interventions.[6] The HNL homodimer ELISA is intended for Research Use Only (RUO).

Principle of the procedure

HNL Homodimer ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the HNL molecule. During the first incubation, HNL in the sample reacts with anti-human HNL antibodies bound to microplate wells. After washing, peroxidase conjugated anti-human HNL antibodies are added. Followed by a second incubation and a simple washing step, the bound conjugate is detected by reaction with 3,3',5,5' tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5%, Sulfuric acid.

The Stop Solution is labelled:

Danger
H318 – Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reusing.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

The Assay Diluent 1X, Wash Buffer, Enzyme Conjugate Buffer, and Calibrator Stock contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

The Assay Diluent 1X, Wash Buffer, Enzyme Conjugate Buffer, and Calibrator Stock are labelled:

Warning
H317 – May cause an allergic skin reaction.
P261 – Avoid breathing vapor.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P280 – Wear protective gloves.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with local, regional, national and international regulations.

Material required but not provided

- Pipettes with appropriate volumes (multichannel for sample transfer, and repeating or multichannel pipettes for addition of Enzyme Conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Reagent reservoirs
- Microplate reader with 450 nm filter
- Microplate orbital shaker (700-900 revolutions per minute)
- Microplate washing device with overflow function (recommended but not required)

Reagents

HNL Homodimer ELISA (10-1380-01) contains reagents for 96 wells, sufficient for 40 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8°C.

Reagent	Amount	Volume	
Coated Plate Mouse monoclonal anti-HNL	1 Plate 8-well strips	96 wells see below	Ready for Use
Transient Plate	1 Plate	96 wells	Ready for Use
Calibrator Stock HNL Antigen Color coded yellow	1 vial	1000 µL	Lyophilized Reconstitute with 500 µL redistilled water and 500 µL Assay Diluent 1X, vortex thoroughly.
Assay Diluent 1X Color coded yellow	1 bottle	50 mL	Ready for Use
Enzyme Conjugate 11X Mouse monoclonal anti-HNL	1 vial	1.1 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 bottle	11 mL	Ready for Use
Wash Buffer 21X Storage after dilution 2–8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make Wash Buffer 1X solution
Substrate TMB Colorless solution Note! *Light sensitive!*	1 bottle	22 mL	Ready for Use
Stop Solution *0.5 M H_2SO_4*	1 vial	7 mL	Ready for use

Preparation of enzyme conjugate 1X solution
Prepare the needed volume of Enzyme Conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all the blue Enzyme Conjugate Buffer into the Enzyme Conjugate 11X bottle. Mix gently.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1.1 mL (1 vial)	11 mL (1 bottle)
8 strips	0.7 mL	7 mL
4 strips	0.35 mL	3.5 mL

Preparation of calibrators

Step 1: Reconstitute the Calibrator Stock

1. Add 500 µL redistilled water and 500 µL Assay Diluent 1X to the calibrator vial.
2. Let the solution stand for 15 minutes at room temperature.
3. Gently invert the vial a few times, then vortex to mix thoroughly.
4. This is the highest concentration calibrator (Calibrator 7; concentration 5.0 µg/L).

Note! If needed, store reconstituted Calibrator 7 at +2-8°C and use the kit within 4 weeks.

Step 2: Prepare serial dilutions

1. Label 6 tubes (Calibrator 1 - 6).
2. Add 300 µL of Assay Diluent 1X to each of these tubes.
3. Perform serial dilutions as follows:
   a. Transfer 200 µL from Calibrator 7 (Stock solution) to Calibrator 6 tube, mix well.
   b. Transfer 200 µL from Calibrator 6 tube to Calibrator 5 tube, mix well.
   c. Repeat this process through Calibrator 1.
4. Calibrator 0 will be the Assay Diluent 1X which serves as the negative control.

Note! See table below for an example of the calibrators' concentrations after the dilutions:

Calibrator	Concentration (µg/L)	Comments
Calibrator 0	0.0	Negative Control
Calibrator 1	0.02	
Calibrator 2	0.051	
Calibrator 3	0.128	
Calibrator 4	0.32	
Calibrator 5	0.8	
Calibrator 6	2.0	
Calibrator 7	5.0	Stock

Specimen collection and handling
The recommended sample type for HNL determinations is EDTA plasma. It is suggested to collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Test procedure
Prepare a calibrator curve for each run. All reagents and samples must be brought to room temperature before use.

1. Add 150 µl calibrators in duplicate to the Transient 96-well Plate.
2. Add 5 µL of each sample into the corresponding wells of the Transient 96-well Plate. Then, add 245 µL of Assay Diluent 1X to each sample to achieve a 1:50 dilution. Shake the plate for 5 seconds to ensure mixing. Note: the final volume (250 µL) is enough for duplicates. If preferred, 2 different wells with the same sample can be prepared.
3. Transfer 100 µL of each calibrator and sample to the Coated 96-well Plate using a multichannel pipette.
4. Incubate on a plate shaker (700-900 rpm) for 60 minutes at room temperature (18-25°C).

5. Wash the wells 6 times x 700 µL using an automatic plate washer with overflow wash function. The chosen program should fill all the wells with wash buffer in each cycle and ensure that the wells are never left without wash buffer (e.g. Plate Mode). Do not use additional soak. Invert and tap the plate firmly against absorbent paper after the final wash.
   Or manually,
   Discard the reaction volume by inverting the microplate over a sink. Perform washes with a multichannel/repeater pipette adding 250 µL of wash buffer to each well and afterwards inverting the microplate over a sink. Repeat this process 5 times. Avoid prolonged soaking during washing procedure.
   Or,
   Hold the plate vertically over a sink and fill the wells by spraying wash buffer into the wells with a wash bottle. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.

   For more information see Technical Note No: 34-0106 Instruction for manual washing procedure for microplates (available online).

6. Add 100 µL of Enzyme Conjugate 1x solution to each well.

7. Incubate on a plate shaker (700-900 rpm) for 60 min at room temperature (18-25°C).

8. Wash as in step 5.

9. Add 100 µL of the Substrate TMB to each well.

10. Incubate for 15 min, in the light, at room temperature (18-25°C) without shaking.

11. Add 50 µL of the Stop Solution to each well and shake the plate for 5 sec.

12. Read optical density at 450 nm and calculate results. Read the plate within 20 min after stopping the reaction.

Note! Be extra careful not to contaminate the Substrate TMB with Enzyme Conjugate solution.

Internal quality controls

Internal plasma pools with low, intermediate and high HNL concentrations should routinely be assayed as samples and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, OD values for the blank, calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculation of results

The concentration of HNL homodimer is obtained by plotting the absorbance of the calibrators, versus their concentration. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. Note that weighting of the curve fit is important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide.

The HNL Homodimer ELISA is validated using Magellan software (Tecan) with Five Parameter Logistic (5PL) and automatic weighting using $1/Y^2$.

Example of results

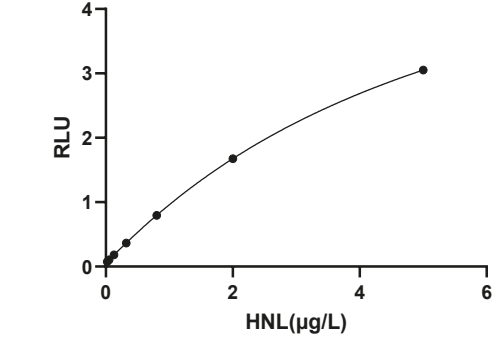
Wells	Identity	Mean $A_{450\ nm}$	Mean conc. µg/L
1A-D	Blank	0.0535	
1E-H	Calibrator 1	0.0757	
2A-D	Calibrator 2	0.1057	
2E-H	Calibrator 3	0.181	
3A-D	Calibrator 4	0.3649	
3E-H	Calibrator 5	0.7954	
4A-D	Calibrator 6	1.6753	
4E-H	Calibrator 7*	3.0516	
6A-D	Sample 1**	0.165	0.11204
5E-H	Sample 2**	0.3857	0.34082
5A-D	Sample 3**	2.6967	4.0378

*Concentration stated on vial label

**Mean concentration measured in the 1:50 sample

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Limitations of procedure

The assay can give higher variations at low and high concentrations of the measuring range. If the sample concentration is in the higher range, some dilutions could be needed. Adjust the dilutions from 1:50 (recommended) to 1:100. If the sample concentration is in the lower range of detection, a lower dilution is required (e.g., 1:10).

Expected values

Good practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own.

Performance characteristics

Precision

Each EDTA plasma sample was analyzed in 4 replicates on 3 different occasions.

		Coefficient of variation	
Sample	Mean value µg/L	Repeatability %*	Within laboratory %**
Sample 1	0.12	3.3	6.2
Sample 2	0.35	3.0	3.6
Sample 3	4.20	5.6	5.9

*Within assay variation

**Total assay variation

Parallellism
10 samples within medium and high concentration of endogenous homodimeric HNL were diluted 1/10, 1/25, 1/50, 1/75 and 1/100; and two laborants have performed the analyses.

For parallelism, precision between samples within dilution series was: 1,3 – 6,6% < 30%.

Calibration
No international reference is available for this assay. The HNL Homodimer ELISA is calibrated in micrograms per liter (µg/L).

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

References

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6. Venge, P., Xu, S., & Mårtensson, J. (2022). HNL Dimer- A Specific Biomarker of Neutrophil Activity - In Sepsis and Antibiotics Monitoring. A Mini-Review. Archives of Clinical and Biomedical Research.

Summary of protocol sheet
HNL Homodimer ELISA

Add calibrator to Transient Plate	150 µL
Dilute samples (1:50) in the Transient Plate	5 µL sample + 245 µL Assay Diluent 1X Shake for 5 sec to ensure mixing
Transfer calibrators and samples to Coated Plate	100 µL with multichannel pipette
Incubate	60 minutes at 18-25°C on a plate shaker (700–900 rpm)
Wash plate with Wash Buffer 1X solution	700 µL, 6 times
Add Enzyme Conjugate 1X solution	100 µL
Incubate	60 minutes at 18-25°C on a plate shaker (700–900 rpm)
Wash plate with Wash Buffer 1X solution	700 µL, 6 times
Add Substrate TMB	100 µL
Incubate	15 minutes at 18-25°C without shaking
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure A450 nm	Read within 20 minutes

For full details see page 9-10

For technical support please contact: support@mercodia.com

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