Product information

User´s Manual

Lactoferrin ELISA

For the determination of lactoferrin in stool.
For research use only, not for use in diagnostic procedures.

REF	**ID09035**
Σ	**96**
Storage:	**2-8°C**

Manufactured for:
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1. INTENDED USE

This IBL-America assay is an enzyme immunoassay intended for the determination of lactoferrin in stool. For research use only, not for use in diagnostic procedures.

2. INTRODUCTION

Lactoferrin is a 76 kDa iron-binding glycoprotein which is synthesized and stored in the secondary granules of neutrophils. It is also present in several secretory fluids, such as milk, saliva, tears, and nasal secretions.

Lactoferrin can exist in different polymeric forms ranging from monomers to tetramers; it tends to polymerize especially at high concentrations.

The physiological activities of lactoferrin include regulation of iron homeostasis, innate defense against a broad range of microbial infections, anti-inflammatory activity, regulation of cellular growth and differentiation and protection against cancer development and metastasis.

3. MATERIAL SUPPLIED

Label	Kit components	Quantity
PLATE	Microtiter plate	12 x 8 wells
WASHBUF	ELISA wash buffer concentrate, 10x	2 x 100 ml
IDK Extract®	Extraction buffer concentrate *IDK Extract*®, 2.5x	1 x 100 ml
CONJ	Conjugate concentrate (rabbit anti human lactoferrin)	1 x 200 µl
STD	Standards, lyophilized	4 x 5 vials
CTRL 1	Control, lyophilized (see specification for range)	4 x 1 vial
CTRL 2	Control, lyophilized (see specification for range)	4 x 1 vial

Label	Kit components	Quantity
SUB	Substrate (tetramethylbenzidine), Ready-to-use	1 x 15 ml
STOP	Stop solution, Ready-to-use	1 x 15 ml
SAMPLEBUF	Sample dilution buffer, Ready-to-use	1 x 50 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra-pure water*
- Stool sample application system (if more information is needed please inquire with IBL-America)
- Calibrated precision pipettors and 10-1000 µl single use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel dispenser or repeating dispenser
- Vortex mixer
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* IBL-America recommends the use of Ultra Pure 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. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at 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 ultrapure water), mix well. Crystals could occur due to high salt concentration in the in the concentrate. Before dilution, the crystals have to be re-dissolved 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**.

- **Preparation of the extraction buffer:** The **extraction buffer concentrate *IDK Extract*®** must be diluted with ultrapure water **1:2.5** before use (100 ml IDK Extract® + 150 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 37°C in a water bath. The ***IDK Extract*®** is stable at **2 - 8 °C** until the expiry date stated on the label. Extraction buffer (1:2.5 diluted IDK Extract®) can be stored in a closed flask at **2 - 8 °C for 4 months**.
- The **lyophilized standards** (STD) and **controls** (CTRL) are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specifications data sheet**. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C or -20 °C for 7 days.**
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in **wash buffer** (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is 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. SPECIMEN COLLECTION AND PREPARATION

Sample stability and storage

Raw Stool

Raw stool is stable for 3 days at room temperature (15-30 °C), 4 days at 2-8 °C or up to 6 months at -20 °C.

Stool extract

Stool extract is stable for 9 days at room temperature (15-30 °C), at 2-8 °C or at -20 °C. Avoid more than three freeze-thaw cycles.

Extraction of the stool samples

Diluted extraction buffer *IDK Extract*® is used as a sample extraction buffer. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instruction for use

Please note that the dilution factor of the final stool suspension depends on the used amount of stool sample and the volume of the buffer.

SAS with 1.5 ml buffer:

Applied amount of stool:	15 mg
Buffer Volume:	1.5 ml
Dilution factor:	1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenization using an applicator, inoculation loop or similar device.

b) Fill the **empty sample tube** with **1.5 ml** of ready-to-use *IDK Extract*® extraction buffer before using it with the sample. **Important:** Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:100

Dilution of samples

The supernatant of the sample preparation procedure (dilution I) is diluted **1:10 in sample dilution buffer**. For example:

- **50 µl** supernatant (dilution I) + **450 µl** sample dilution buffer, mix well = **1:10** (**dilution II**). This results in a final dilution of 1:1000.

100 µl of **dilution II** per well are used in the test.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is intended for the determination of lactoferrin in stool. In a first incubation step, the lactoferrin in the samples is bound to polyclonal antibodies, immobilized to the surface of the microtiter wells. To remove all unbound substances, a washing step is carried out. In a second incubation step, a peroxidase-labeled conjugate (rabbit anti human lactoferrin) is added which recognizes specifically the bound lactoferrin. After another washing step to remove all unbound substances, the solid phase is incubated with the substrate, tetramethylbenzidine (TMB), which reacts with the peroxidase. An acidic stop solution is added to stop the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of lactoferrin. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated using the values obtained from the standards. Lactoferrin 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 the standards/controls/samples on a protocol sheet.

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 IBL-America.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminum packaging at 2-8 °C. Strips are stable until expiry date stated on the label.

We recommend to pipet the standards and controls in duplicate.

1.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
2.	Cover the strips and incubate for **30 min** at room temperature (15–30 °C).
3.	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 tapped on absorbent paper.
4.	Add **100 µl conjugate** in each well.
5.	Cover the strips and incubate for **30 min** at room temperature (15–30 °C).
6.	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 tapped on absorbent paper.
7.	Add **100 µl** substrate (SUB) in each well.
8.	Incubate for **10–20 min*** at room temperature (15–30 °C) in the **dark**.
9.	Add **100 µl stop solution** (STOP) into each well and mix well.
10.	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.

* The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop 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 calibrator 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 pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Stool samples

The obtained results have to be multiplied with the **dilution factor of 1000** to get the actual concentrations.

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

LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can 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 determined.

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:

LoB × *sample dilution factor to be used*

LoB see chapter “Performance Characteristics”.

10. QUALITY CONTROL

IBL-America recommends the use of external controls for internal quality control, if possible.

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

Reference range

We recommend each laboratory to establish its own reference concentration range.

11. PERFORMANCE CHARACTERISTICS

Accuracy-Precision

Repeatability (Intra-Assay); n = 40

Sample	Mean Value [µg/ml]	CV [%]
1	28.75	4.1
2	6.56	4.8

Reproducibility (Inter-Assay); n = 16

Sample	Lactoferrin [µg/ml]	CV [%]
1	17.66	9.5
2	4.45	14.0

Analytical Sensitivity

The following values have been estimated based on the cocntrations of the standards without considering possibly used sampled dilution factors.

Limit of blank, Lob 0.222 ng/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only, not for use in diagnostic procedures.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- 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 out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not to assemble wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colorless 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 the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- 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. IBL-America can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints in respect of deficiencies must be logged within 14 days after receipt of the product. The product should be sent to IBL-America along with a written complaint.

15. REFERENCES

1. Levay, P. F. & Viljoen, M. Lactoferrin: a general review. Haematologica 80, 252–67 (1995).
2. Gisbert, J. P., McNicholl, A. G. & Gomollon, F. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. Inflammatory bowel diseases 15, 1746–54 (2009).
3. Uchida et al. Immunochemical detection of human lactoferrin in feces as a new marker for inflammatory gastrointestinal disorders and colon cancer. Clin. Biochem. 27, 256-64 (1994)