ab285281 – Mouse Lactoferrin ELISA Kit

For quantitative measurement of Lactoferrin in mouse serum, plasma or other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285281

Storage and Stability

An unopened kit can be stored at 4°C for 1 month.

If the kit is not used within 1 month, store the items separately according to the table below once the kit is received.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	1	-20°C
Standard	2 vials	-20°C
Sample and Standard diluent	3 vials	4°C
100X Biotinylated detection antibody	120 µl	-20°C
Biotinylated Detection Ab Diluent	14 ml	4°C
100X HRP Conjugate	120 µl	-20°C
HRP Conjugate Diluent	14 ml	4°C
X25 Wash buffer	30 ml	4°C
Substrate Reagent	10 ml	4°C
Stop Solution	10 ml	4°C
Plate sealers	4	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader with 450 nm wavelength filter
- Deionised and distilled water

Reagent Preparation

Δ Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

HRP Conjugate: Calculate the required amount before the experiment (100 µl/well). Dilute the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent.

Biotinylated Detection Antibody: Calculate the required amount before the experiment (100µl/well). Centrifuge the stock tube before use; dilute the 100x Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.

Wash Buffer: Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. If crystals are present, warm it in a 40°C water bath and mix gently until the crystals are completely dissolved.

Standard Preparation

1) Centrifuge the standard at 10,000xg for 1 min.
2) Add 1.0 ml of Standard and Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 4000 pg/mL.
3) Then make serial dilutions as needed. The recommended dilution gradient is as follows: 4000, 2000, 1000, 500, 250, 125, 62.5, 0 pg/ml.
4) Prepare 7 tubes, add 500 µl of Standard & Sample Diluent to each tube.
5) Pipette 500 µl of the 4000 pg/ml stock solution to the first tube and mix up to produce a 2000 pg/ml working solution.
6) Transfer 500 µl of the solution into the other tube to form 2- fold serial dilutions of the highest standards to make the standard curve within the range of this assay

Sample Preparation

- Samples should be assayed within 7 days when stored at 4°C, otherwise aliquot and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 15 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be endotoxin free.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000xg at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000xg. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1x106 cells, add 150-250 µl of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500xg at 4°C. Remove the cell fragments; collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000xg to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 min at 1000xg at 2~8°C. Collect the supernatant to carry out the assay.

Assay Protocol

Δ Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1) Add 100 µl of each standard or samples into appropriate wells.
2) Cover the plate with the plate sealer and incubate for 90 min at 37°C.
   Δ Note: solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and bubble formation.
3) Remove the liquid from each well, do not wash. Immediately add 100 µl of Biotinylated Detection Antibody working solution to each well. Cover with the plate sealer. Gently mix and incubate for 1 hour at 37°C.
4) Aspirate the solution from each well and add 350 µl of 1x wash buffer to each well. Leave it for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

Δ **Note:** a microplate washer can be used for all wash steps.

5) Add 100 µl of HRP Conjugate working solution to each well. Cover with plate sealer and incubate for 30 min at 37°C.
6) Aspirate the solution from each well, repeat the wash process for five times as described in step 4
7) Add 90 µl of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light.
   Δ **Note:** the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
8) Add 50 µl of Stop Solution to each well.
   Δ **Note:** the stop solution should be added in the same order as the substrate solution.

Measurement

Read absorbance in micro plate reader at 450 nm.

Calculation

- Determine the average of the duplicate readings for each standard and sample.
- Subtract the average blank standard from other samples.
- Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis.
- If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample is above the upper limit of the standard curve, retest the samples with appropriate dilution.
- The actual concentration is the concentration obtained after calculation multiplied by the dilution factor.

Technical Support

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