

ab200015 – Human Lactoferrin SimpleStep ELISA® Kit

For the quantitative measurement of Lactoferrin in human serum, plasma, milk, urine, saliva, and cell culture supernatant.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab200015

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1x96 tests	Quantity 10x96 tests	Storage Condition
Human Lactoferrin Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Lactoferrin Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Lactoferrin Lyophilized Purified Protein	2 Vials	10 x 2 vials	+4°C
Antibody Diluent 5BI	6 mL	10 x 6 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
TMB Development Solution	12 mL	120 mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

1X Wash Buffer PT:

Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail:

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

Standard Preparation

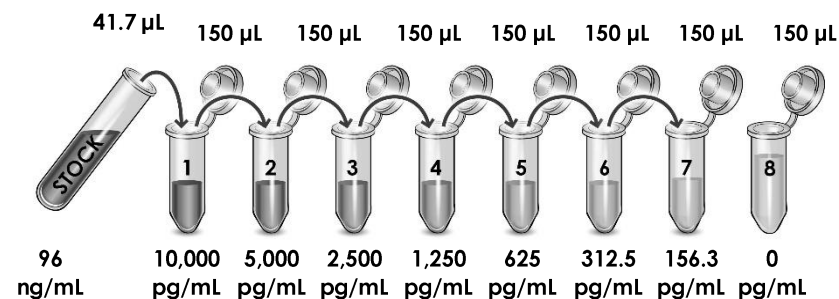
Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the Lactoferrin standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Lactoferrin standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 96 ng/mL **Stock Standard** Solution.

Label eight tubes, Standards 1–8.

Add 358.3 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	Range
Serum	1:6,400 – 1:200
Plasma - Citrate	1:6,400 – 1:200
Plasma - Heparin	1:6,400 – 1:200
Plasma - EDTA	1:3,200 – 1:200
Milk	1:32,000,000 – 1:500,000
Urine	1:1,280 – 1:20
Saliva	1:320,000 – 1:10,000
RPMI 1640 Cell Culture Media*	≤100%

*Based on spiked sample

Plasma

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:200 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:20 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva

Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:10,000 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Milk

De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples at least 1:500,000 into Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate

- 1) Prepare all reagents, working standards, and samples as directed in the previous sections.
- 2) Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
- 3) Add 50 µL of all sample or standard to appropriate wells.
- 4) Add 50 µL of the Antibody Cocktail to each well.
- 5) Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

- 6) Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
- 7) Add 100 µL of TMB Development Solution to each well and incubate for 8 minutes in the dark on a plate shaker set to 400 rpm. *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
- 8) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
- 9) Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength	600 nm
Time	up to 20 min
Interval	20 sec - 1 min
Shake	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus