

One-Step Fast Human Ferritin ELISA

For the quantitative determination of human Ferritin concentrations in serum, urine and plasma.

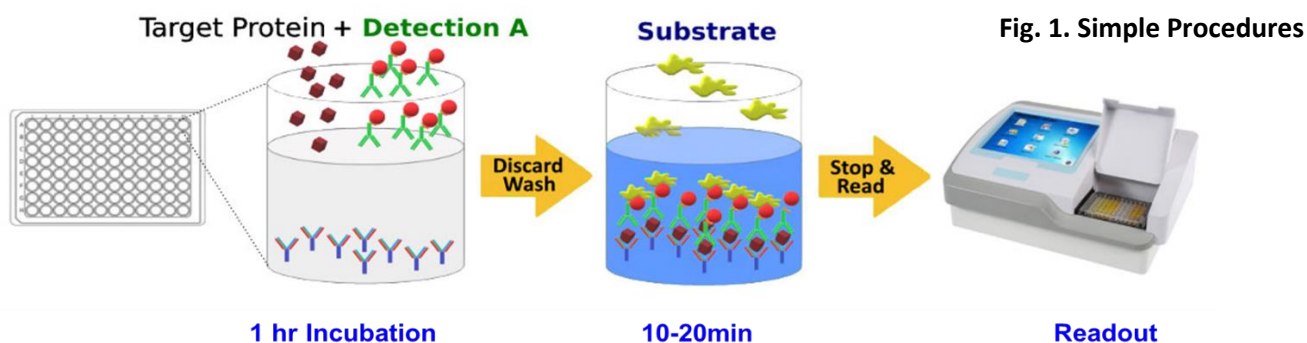
INTRODUCTION

Ferritin is the universal intracellular iron-storage protein composed of 24 subunits of the heavy and light ferritin chains, acting as a buffer against iron deficiency and iron overload. Ferritin is found in most tissues as a cytosolic protein while small amounts are secreted into the serum where it functions as an iron carrier. Plasma ferritin is also an indirect marker of the total amount of iron stored in the body, hence, serum ferritin is used as a diagnostic test for iron-deficiency anemia. Ferritin participates in ferroxidase activity, immune response, Stress response, etc.

Tribioscience’s Fast Human Ferritin ELISA is designed to quantitatively detect human Ferritin levels in serum, plasma, and other biological samples. The main feature is that **the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate, and fast. The measurement can be finished in 1 hour, not need 4-5 hours (Fig. 1). The detection range is from 0.3 to 243 ng/mL.** The levels of human Ferritin samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human Ferritin protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human Ferritin was pre-coated onto a microplate. Standards or samples, and HRP-conjugated Ferritin antibody are pipetted into the wells and incubated together. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate read at 450.



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human Ferritin Microplate	TBS3200A	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Ferritin.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human Ferritin Standard	TBS3200B	100 µl of Recombinant human Ferritin (2.43ug/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3200C	2.1 ml of HRP- human Ferritin antibody.	May be stored for up to 3 months at 2-8 °C.
Assay Diluent	TBS3200E	12 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution(10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration dates.

The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

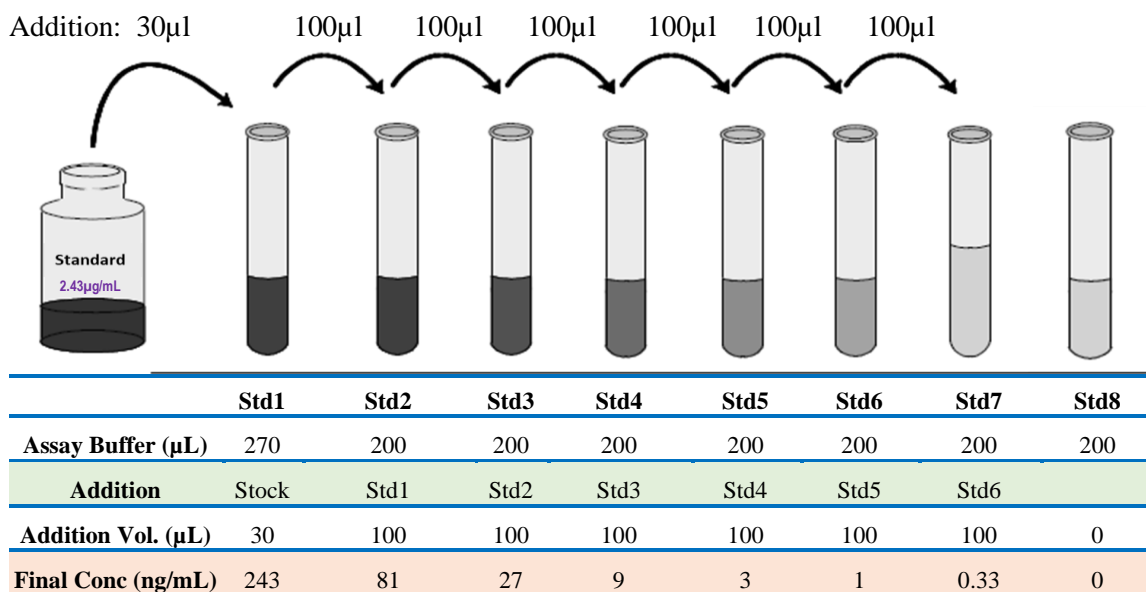
Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved*).

Human Ferritin Standard Preparation: Label test tubes as #1 through #8. Pipet 270 μL of 1x Assay Diluent into tube #1, and 200 μL into tubes #2 to #8 **as diagram below**.

1. Add 30 μL of the Human Ferritin Standard stock solution (2.43 $\mu\text{g}/\text{mL}$) to tube #1 and mix.
2. Make 3x serial dilutions of the standard using the Tube#1(243 ng/mL standard solution) from Tube #2 through #7 with sequential transfer of 100 μL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 243, 81, 27, 9, 3, 1, and 0.33 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human Ferritin standard preparation



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 μL of standard, sample, or control per well.
2. Add 20 μL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 1 hour**.
3. Aspirate each well, and wash for 3 times by filling each well with 200 μL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 μL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve ($R^2=0.999$) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human is typically 1ng/ml. The Intra-assay CV and the Inter-assay CV are <10%.

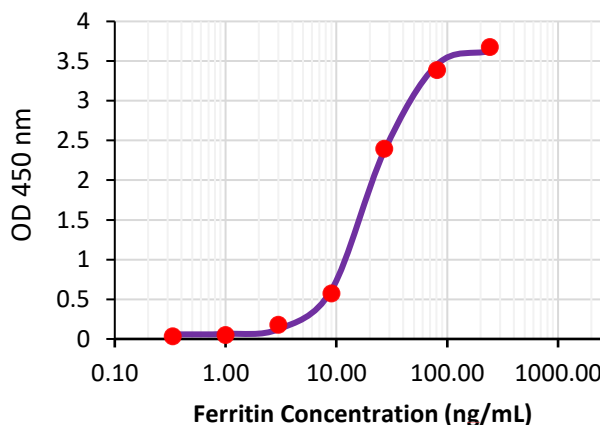
SPECIFICITY

This assay recognizes natural and recombinant human Ferritin. No cross-reactivity with others.

RELATIVE PRODUCTS

- Human IL-1 β ELISA (TBS3219)
- Human IL-2 ELISA (TBS3220)
- Human IL-4 ELISA (TBS3221)
- Human IL-6 ELISA (TBS3223)
- Human IL-7 ELISA (TBS3224)
- Human IL-8 ELISA (TBS3225)
- Human IL-10 ELISA (TBS3226)
- Human IL-13 ELISA (TBS3227)
- Human IL-17 ELISA (TBS3228)
- Human IL-22 ELISA (TBS3229)
- Human IL-33 ELISA (TBS4245)
- Human VASN ELISA (TBS4246)
- Human IFN-gamma ELISA (TBS3230)
- Human TGF- β 1 ELISA (TBS3232)
- Human GM-CSF ELISA (TBS3233)
- Human MIP-1 α ELISA (TBS3234)
- Protein Cell Lysis Buffer (catalog# TBS5001)
- Protein Assay Kit (Catalog# TBS2005)
- TMB Substrate System (Catalog#TBS5021)

Figure 3. Human Ferritin Standard Curve



For research use only. Not for use in diagnostic procedures.