EAGLE BIOSCIENCES

Rat Ferritin ELISA Assay Kit

Catalog Number:
RFE21-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 4.1

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INTENDED USE

The Eagle Biosciences Rat Ferritin ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the measurement of Ferritin in rat biological samples. The Eagle Biosciences Rat Ferritin ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY BACKGROUND

Ferritin is a key intracellular iron-storage protein that plays a crucial role in maintaining iron homeostasis. In Rattus norvegicus (rat), as in humans, ferritin sequesters excess iron in a non-toxic form, releasing it in a controlled manner to support essential physiological processes such as oxygen transport, DNA synthesis, and cellular respiration. Ferritin is composed of 24 subunits of heavy (H) and light (L) chains, and its expression is regulated in response to iron levels, oxidative stress, and inflammatory stimuli

In preclinical research, ferritin levels in rat models are commonly used as a biomarker for systemic iron status, inflammation, and iron-related pathologies such as anemia of chronic disease or hemochromatosis. Because of its sensitivity to both iron availability and immune activation, serum ferritin is frequently measured in toxicology and pharmacology studies to assess drug-induced effects on iron metabolism or liver function. Additionally, brain ferritin is studied in neurological models to explore iron dysregulation in conditions such as Parkinson's and Alzheimer's disease.

Clinically relevant insights can also be derived from ferritin measurements in translational research. Rat models provide a bridge between molecular mechanisms and therapeutic targets in humans, particularly for diseases involving iron overload or deficiency. Ferritin assays are often used in biomarker validation studies for diagnostics or treatment monitoring, supporting the development of iron-modulating therapies and anti-inflammatory agents.

PRINCIPLE OF THE ASSAY

In this assay the Ferritn present in samples reacts with the anti-Ferritin antibodies, which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-Ferritin antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound Ferritin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Ferritin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Ferritin in the test sample. The quantity of Ferritin in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

LIMITATIONS RELATED TO INTENDED USE

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.

- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 0.3M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type

and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.

- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens, All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing. If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- Serum samples - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Plasma samples - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80°C (preferably) or -20° C. Avoid repeated freeze-thaw cycles.
- Urine samples - Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

Specimen Pre-Treatment

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- Serum and Plasma Samples - Recommended starting dilution is 1:40. To prepare a 1:40 dilution of a sample, transfer 10 µL of sample to 390 µL of 1X diluent. This gives you a 1:40 dilution. Mix thoroughly at each stage.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtiter washer/aspirator
- Distilled or Deionized H2O
- Microtiter Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

REAGENTS PROVIDED

1. **Microplate**

Contents:	One plate of 12 removable 8 well strips, antibody coated in a sealed foil bag with desiccant.
Format:	Ready to Use
Storage:	2-8°C
Stability:	Stable until the expiry date printed on the label

2. **Enzyme-Antibody Conjugate (100x)**

Contents:	One bottle containing Horseradish Peroxidase Conjugated antibody in a stabilizing buffer
Format:	Concentrated; Requires Preparation
Volume:	150 µL/bottle
Storage:	2-8°C in the dark
Stability:	Stable until the expiry date printed on the label. After preparation, use immediately and discard any reagents left over.
Preparation of Enzyme-Antibody Conjugate:	**Immediately prior to use dilute 1:100.** Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Protect from light. Mix uniformly, but gently. Avoid foaming.

3. **Standard Concentrate**

Contents:	One bottle of lyophilized standard
Format:	Lyophilized; Requires Preparation and Aliquation
Storage:	2-8ºC for lyophilized standard. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles.

Stability:	Stable until the expiry date printed on the label. After preparation, use immedietly and discard any reagents left over.
Preparation of working solution:	Prepare according to the lot specific Certificate of Analysis.

4. **Diluent Solution Concentrate (5x)**

Contents:	One bottle of concentrated diluent buffer
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8ºC for both 1X working solution and 5x concentrate
Stability:	Stable until the expiry date printed on the label
Preparation of Working Solution:	**Dilute 1:5** with distilled or deionized water. (1 part buffer concentrate to 4 parts water)

5. **Wash Solution Concentrate (20x)**

Contents:	One bottle of 20X wash solution
Format:	Concentrated; Requires Preparation
Volume:	50 mL/bottle
Storage:	2-8ºC for both 1X working solution and 20X concentrate
Stability:	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentrate is stable until the expiration date.
Preparation of Working Wash Solution:	**Dilute 1:20** with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35ºC before dilution can dissolve crystals.

6. **TMB Substrate Solution**

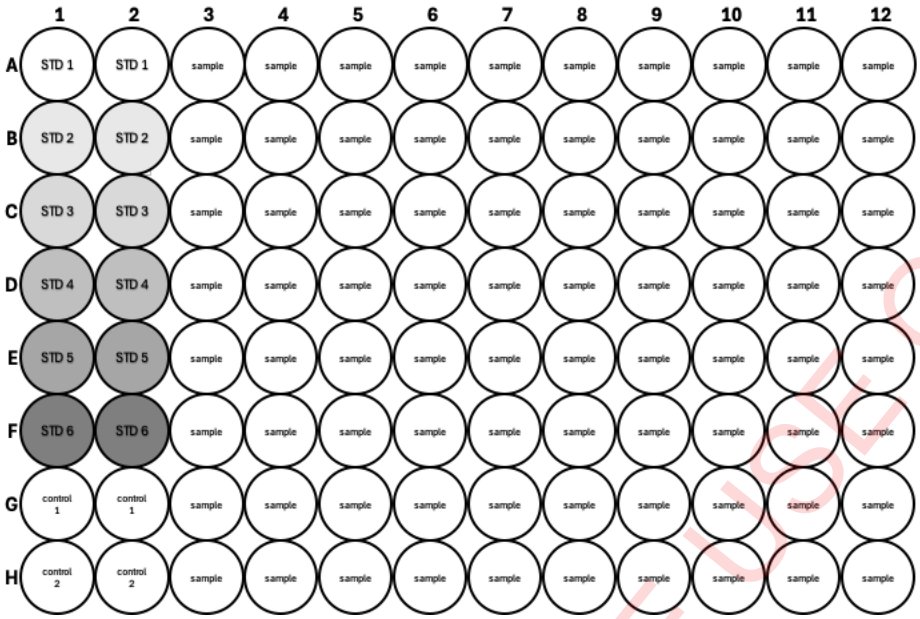
Contents:	One bottle of 3,3',5,5'- tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at PH 3.3.
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C in the dark
Stability:	Protect from light. Stable until the expiration date printed on the label

7. **Stopping Solution**

Contents:	One bottle of 0.3 M sulfuric acid
Format:	Ready to Use
Volume:	12 mL/bottle
Storage:	2-8°C
Stability	Stable until the expiry date printed on the label.

Safety	Refer to product SDS.

RECOMMENDED ASSAY LAYOUT*



***** *Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

Note: The standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

1. Pipette 100 µL of the prepared standards and samples into pre-designated wells.
2. Incubate the microtiter plate at room temperature for 60 minutes. Keep plate covered and level during incubation.
3. Following incubation, aspirate the contents of the wells.
4. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
5. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for 10 minutes. Keep plate covered in the dark and level during incubation.
6. Wash and blot the wells as described in Steps 3/4.
7. Pipette 100 µL of TMB Substrate Solution into each well.
8. Incubate in the dark at room temperature for precisely ten (10) minutes.
9. After ten minutes, add 100 µL of Stop Solution to each well.
10. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

CALCULATIONS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the Ferritin concentration in original samples.

QUALITY CONTROL

The test results are only valid if the test has been performed following the following instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards/controls must be found within the acceptable ranges as stated above and/or labeled. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation, the following technical issues should be reviewed. Expiration dates of prepared reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.