BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access Ferritin

REF 33020
C22490

FOR PROFESSIONAL USE ONLY

For *in vitro* diagnostic use

Rx Only

For use on DxI Access Immunoassay Analyzers

PRINCIPLE

INTENDED USE

The Access Ferritin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of ferritin levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

Ferritin is used as an aid in the diagnosis of iron deficiency or iron overload.[1,2]

SUMMARY AND EXPLANATION

Ferritin is a large spherical protein consisting of 24 non-covalently linked subunits and having a molecular weight of approximately 450,000.[3] The subunits form a shell surrounding a central core containing variable amounts of ferric hydroxyphosphate.[4] One molecule of ferritin is capable of binding between 4,000 and 5,000 atoms of iron, making ferritin a major iron storage protein in tissues.[5]

Found chiefly in the cytoplasm of cells of the reticuloendothelial system, it once was thought that ferritin did not appear in plasma or extracellular fluid under normal conditions. However, the development of a sensitive immunoradiometric technique by Addison, et al., in 1972, resulted in the discovery that ferritin is a constituent of all normal human serum.[6] Through this and other work, it was determined that the concentration of ferritin was directly proportional to the total iron stores in the body, resulting in serum ferritin levels becoming a common diagnostic tool in the evaluation of iron status.[2]

In most normal adults serum ferritin levels ranges from 10-300 ng/mL (µg/L), but concentrations vary with age and sex.[2,3] There is a sharp rise in serum ferritin levels in the first month of life, coinciding with the depression of bone marrow erythropoiesis.[3] Within two or three months, erythropoiesis becomes reactivated and there is a drop in the concentration of serum ferritin. By six months, the concentration is reduced to fairly low levels where they remain throughout childhood. There is no sex difference until the onset of puberty, at which time ferritin levels rise, particularly in males.[3] There is a significant positive correlation between age and serum ferritin levels in females, but not in males.[7]

Addison, et al., found that patients with iron deficiency anemia have serum ferritin levels approximately one tenth of normal subjects, while patients with iron overload (hemochromatosis, hemosiderosis) have serum ferritin levels much higher than normal.[6] Other studies also suggest that serum ferritin levels provide a sensitive means of detecting iron deficiency at an early stage.[4,7,8] In addition, serum ferritin is widely recognized as an acute phase reactant and marker of acute and chronic inflammation, and is nonspecifically elevated in a wide range of inflammatory conditions.[2]

Traditionally, the estimation of stainable iron in bone marrow biopsies was the accepted method for the evaluation of body iron stores. However, this method is traumatic for the patient and only semi-quantitative. Other methods, such as serum iron determination, total iron binding capacity (TIBC) and percent saturation of transferrin are subject to diurnal variations and are often imprecise. These latter methods also do not discriminate between depleted iron stores and

conditions associated with defective iron release (eg. anemia of chronic disease).[9] The Access Ferritin assay is based on the two-site immunoradiometric assay (IRMA) described by Addison, et al., but utilizes an enzyme labeled antibody in place of the radiolabeled tracer.[6] The measurement of ferritin is very well suited to this assay method as its very large size easily permits the simultaneous binding of the required two (or more) antibodies.

METHODOLOGY

Assay type: one-step, sandwich

The Access Ferritin assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel with goat anti-ferritin-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-mouse: mouse anti-ferritin complexes. Serum or plasma (heparin) ferritin binds to the immobilized monoclonal anti-ferritin on the solid phase, while the goat anti-ferritin enzyme conjugate reacts with different antigenic sites on the ferritin molecules.

After incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of analyte in the sample. Analyte concentration is automatically determined from a stored calibration.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[10]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than 8 hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Avoid assaying grossly hemolyzed samples in that ferritin can be released from lysed red cells.

REAGENTS

CONTENTS

Access Ferritin Reagent Pack

Ref. No. 33020: 100 determinations, 2 packs, 50 tests/pack

Ref. No. C22490: 200 determinations, 2 packs, 100 tests/pack

The same reagent formulation is used on all Access Immunoassay Systems.

All antisera are polyclonal unless otherwise indicated.

Well	Contents	Ingredients
R1a:	3.22 mL	Paramagnetic particles coated with goat anti mouse IgG: mouse monoclonal anti-ferritin complexes suspended in TRIS buffered saline, with surfactant, bovine serum albumin (BSA), < 0.1% sodium azide, and 0.1% ProClin* 300.
R1b:	2.98 mL	Goat anti-ferritin-alkaline phosphatase (bovine) conjugate in TRIS buffered saline, with surfactant, BSA, protein (goat, mouse), < 0.1% sodium azide, and 0.1% ProClin 300.

*ProClin is a trademark of LANXESS Corp.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Ferritin Particles (Compartment R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Ferritin Conjugate (Compartment R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Ferritin Calibrators
   Provided at zero and approximately 10, 50, 200, 500 and 1,500 ng/mL (µg/L).
   Ref. No. 33025
2. Quality Control (QC) materials: commercial control material.
3. Lumi-Phos PRO
   Ref. No. B96000

4. UniCel DxI Wash Buffer II
   Ref. No. A16793
5. Optional materials for dilution:
   - Access Sample Diluent A
     - Vial Ref. No. 81908
     - Diluent Pack Ref. No. A79783

REAGENT PREPARATION

Provided ready to use.

REAGENT STORAGE AND STABILITY

Stability	
Unopened at 2 to 10°C	Up to stated expiration date
After opening at 2 to 10°C	28 days

- Store upright.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Signs of possible deterioration are a broken elastomeric layer on the pack or quality control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

CALIBRATION

CALIBRATION INFORMATION

An active calibration is required for all tests. Calibration is required every 28 days. See calibrator Instructions For Use (IFU) for additional calibration information. Refer to the appropriate system manuals and/or Help system for information on calibration method, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials are essential for monitoring the system performance. Quality controls with varying concentration ranges should be run individually at least once every 24 hours when the assay is being performed.[11] Quality control ranges should be determined by each laboratory's individual requirements. Follow applicable regulations and guidelines for quality control.

TESTING PROCEDURE(S)

PROCEDURE

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.
3. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

4. Use 5 µL of sample for each determination in addition to the sample container and system dead volumes. Use 8 µL of sample in addition to the sample container and system dead volumes for each determination run with the automated dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), µg/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 1.

LIMITATIONS

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce human anti-animal antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other antibodies such as human anti-goat antibodies may be present in patient samples.[12,13] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
2. Other potential interferences in the sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, fibrin, endogenous alkaline phosphatase, exogenous alkaline phosphatase (e.g. asfotase alfa, Strensiq), and proteins capable of binding to alkaline phosphatase. Carefully evaluate results if the sample is suspected of having these types of interferences.[14,15]
3. The results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
4. The Access Ferritin assay does not demonstrate any "hook" effect up to 40,000 ng/mL (µg/L). Extremely high quantities of ferritin may cause a "hook" effect.

RESULTS INTERPRETATION

Test results are determined automatically by the system software. Test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

MEASURING INTERVAL

0.6-1,500 ng/mL (µg/L)

Automated dilution: Up to 75,000 ng/mL (µg/L)

Samples can be accurately measured within the measuring interval defined as the lower Limit of Quantitation (LoQ) and the highest calibrator value.

1. If a sample contains less than the lower limit for the assay, report the result as less than that value (i.e. < 0.6 ng/mL [µg/L]).
2. If a sample contains more than the stated value of the highest calibrator, report the result as greater than that value (e.g. > 1,500 ng/mL [µg/L]). Alternatively, the sample may be diluted to obtain a result.
   - For automated dilutions, the system dilutes one volume of sample with 49 volumes of Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions.
   - For manual dilutions, dilute one volume of sample with 49 volumes of Sample Diluent A.

EXPECTED VALUES

1. Each laboratory should validate or establish its own reference intervals to assure proper representation of specific populations.

2. Ferritin concentrations were measured in 113 serum samples from apparently healthy male and female subjects using the Access Ferritin assay. The results were as follows:

	n	Geometric Mean (ng/mL, µg/L)	95% Range† (ng/mL, µg/L)
Males	49	105.6	23.9 - 336.2
Females	64	51.4	11.0 - 306.8

†Non-parametric estimate of 95% confidence interval.

3. Interpret the results of this test in conjunction with the patient's clinical presentation.

PERFORMANCE CHARACTERISTICS

ASSAY CRITERIA AND REPRESENTATIVE DATA

Representative data is provided for illustration only. Performance obtained in individual laboratories may vary.

METHODS COMPARISON

A study based on CLSI EP09c, 3rd Edition[16] using Passing-Bablok regression and Pearson's correlation compared the Access 2 Immunoassay System and the DxI 9000 Access Immunoassay Analyzer.

N	Concentration Range* (ng/mL [µg/L])	Slope	Slope 95% CI	Intercept	Intercept 95% CI	Correlation Coefficient R
147	2.3 - 1471	0.96	0.95 - 0.97	0.23	-0.34 - 1.08	0.99

*Range is Access 2 values

LINEARITY

A study based on CLSI EP06-Ed2[17] performed on the DxI 9000 Access Immunoassay Analyzer determined the assay demonstrated linearity across the measuring interval.

IMPRECISION

The assay was designed to have within-laboratory imprecision as listed below:

- ≤ 0.5 ng/mL (µg/L) SD at concentrations ≤ 5 ng/mL (µg/L)
- ≤ 10.0% CV at concentrations > 5 ng/mL (µg/L)

A study based on CLSI EP05-A3[18] performed on the DxI 9000 Access Immunoassay Analyzer tested multiple samples in duplicate in 2 runs per day for a minimum of 20 days.

Concentration (ng/mL [µg/L])			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	N	Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	80	1.2	0.2	16.5	0.0	0.0	0.1	5.3	0.2	17.3
Sample 2	80	13	0.4	3.1	0.2	1.3	0.4	3.2	0.6	4.7

Concentration (ng/mL [µg/L])			Repeatability (Within-Run)		Between-Run		Between-Day		Within-Laboratory	
Sample	**N**	**Mean**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sample 3	80	147	4.5	3.0	2.0	1.4	6.0	4.1	7.7	5.3
Sample 4	80	289	8.6	3.0	0.0	0.0	9.1	3.1	12.5	4.3
Sample 5	80	560	17.2	3.1	10.0	1.8	16.2	2.9	25.7	4.6
Sample 6	80	1276	53.3	4.2	5.8	0.5	42.2	3.3	68.2	5.3

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 5 mg/dL (86 µmol/L) bilirubin, lipemic samples containing the equivalent of 900 mg/dL (10.16 mmol/L) triglycerides, and hemolyzed samples containing up to 300 mg/dL (3 g/L) hemoglobin do not affect the concentration of ferritin assayed. Grossly hemolyzed samples should not be used in that ferritin can be released from lysed red cells.

Samples containing 5-9 g/dL (50-90 g/L) albumin do not affect the concentration of ferritin assayed.

The antibodies used in this kit were raised against liver ferritin. Reactivity to spleen ferritin, as demonstrated by spiking recovery in a serum sample, is equivalent to that of liver ferritin.

DETECTION CAPABILITY

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies were conducted on the DxI 9000 Access Immunoassay Analyzer following CLSI guideline EP17-A2.[19] The LoB study included multiple reagent lots and instruments over a minimum of 3 days. The LoD and LoQ studies included multiple reagent lots and 3 instruments over a minimum of 5 days.

	ng/mL (µg/L)
Limit of Blank (LoB)	0.2
Limit of Detection (LoD)	0.4
Limit of Quantitation (LoQ) ≤ 20% within-lab CV	0.6

ADDITIONAL INFORMATION

REVISION HISTORY

Revision A

New release of DxI Access Immunoassay Analyzer reagent IFU.

Revision B

Updated "Intended Use" section.

Updated "Limitations" section.

Updated "Measuring Interval" section.

Updated "Methods Comparison" section.

Updated "Imprecision" section.

Updated "Detection Capability" section.

Revision C

Updated ProClin trademark statement.

SYMBOLS KEY

Glossary of Symbols is available at beckmancoulter.com/techdocs (document number C02724).

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