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Diazyme Ferritin Assay

Configuration
The Diazyme Ferritin Assay reagent is provided in bulk and the following kit configuration:

REF	Configuration
DZ526A-K	R1:1 x 27 mL R2: 1 x 8.5 mL

* Calibrators and Controls sold separately

Intended Use
The Diazyme Ferritin Assay is for the quantitative determination of ferritin in human serum, K_2EDTA plasma, and lithium heparin plasma on Hitachi 917 analyzer. For *in vitro* diagnostic use only.

Summary[1-5]
Ferritin is the iron storage protein of the body with a critical role in iron homeostasis. The iron-free protein, apoferritin (MW 450 kDa) consists of 24 subunits, acidic heavy (H, MW 21.0 kDa) and weakly basic light (L, MW 18.5 kDa) monomers, forming a shell to hold ferric iron as ferrihydrite. The basic isoferritins are responsible for long-term iron storage function and are mainly detectable in the human liver, spleen and bone marrow. Serum ferritin is correlated with the available iron storage in body. Ferritin is also used as a marker for iron overload disorders and iron deficiency anemia.

Assay Principle
The Diazyme Ferritin Assay is based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between Ferritin in the sample and anti-Ferritin antibodies conjugated to latex particles, agglutination occurs. This agglutination is detected as an absorbance change (560 nm), with the magnitude of the change being proportional to the quantity of Ferritin in the sample. The actual concentration is then determined from a calibration curve prepared from calibrators of known concentrations.

Reagent – Working Solutions
REAGENT 1: 100 mM Tris buffer solution, ready to use

REAGENT 2: Suspension of latex particles coated with goat anti-human Ferritin antibodies, ready to use.

Precautions
1. For *in vitro* diagnostic use.
2. Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the laws of the State in which he practices, to use or order the use of the device.
3. Do not use the reagents, calibrator, and controls after the expiration date labeled on the outer box.
4. The assay should be recalibrated and controls should be run with each new lot of reagents.
5. Avoid ingestion and contact with skin and eyes.
6. Samples containing precipitates should not be used.
7. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
8. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

Warnings
The REAGENT contains <0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

Reagent Handling
1. R1 ready for use
2. R2 mix reagent gently before use and once weekly thereafter

Reagent Stability and Storage
REAGENT, CALIBRATOR, and CONTROL should be stored at 2-8°C. **DO NOT FREEZE**. The REAGENT, CALIBRATOR, and CONTROL are stable when stored as instructed until the expiration date on the label.

Specimen Collection and Handling
Serum, heparinized plasma, or K_2 EDTA plasma samples can be tested with the Diazyme Ferritin Assay. For serum, collect whole blood by venipuncture and allow clotting. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection.

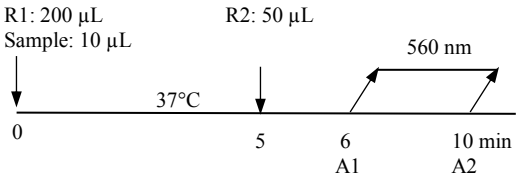
Sample stability[3]: 7 days at 2-8°C; 6 months at -20°C. Avoid repeated freezing and thawing. Do not thaw frozen specimens in a 37 °C bath. Violent mixing may denature ferritin. Samples containing precipitates should not be used

Materials Provided
See "Reagent – Working Solutions" section for reagents.

Materials Required But Not Provided
- CONTROL for validating the performance of the Diazyme Ferritin Assay are provided separately (REF DZ526A-CON)
- CALIBRATOR for the Diazyme Ferritin Assay are provided separately (REF DZ526A-CAL)
- 0.9% saline is used for diluting serum and plasma samples and as a zero CALIBRATOR
- General laboratory equipment

Assay Procedure
As with any diagnostic test it is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.

Below is an assay test scheme for the Hitachi 917 analyzer. Refer to application sheet (70805).



The ferritin concentration in each sample is determined with the read absorbance change from a calibration curve prepared with calibrators of known concentrations. For technical questions, please call 858-455-4768 or email: support@diazyme.com.

Quality Control
We recommend that each laboratory use the Diazyme Ferritin Control Set to validate the performance of the Diazyme Ferritin Assay REAGENT. A set of normal and abnormal ranges of CONTROL is available from Diazyme Laboratories (REF DZ526A-CON). Each laboratory should follow federal, state, and local guidelines for testing QC material.

Calibration
Four levels of CALIBRATOR (REF DZ526A-CAL) are provided separately. For automated analyzers, use saline and the CALIBRATOR 1-4 for calibration. The lot specific CALIBRATOR values are stated in the Certificate of Analysis.

Calibration frequency
The calibration curve is stable for at least 30 days on the Hitachi 917 analyzer. Additionally, the assay should be recalibrated and CONTROL run with each new lot of REAGENT. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Expected Value
Adults: Expected values for ferritin concentrations in clinically healthy subjects are strongly dependent upon age and sex[4]. The reference interval of Diazyme Ferritin Assay was evaluated according to CLSI C28-A3 protocol with serum specimens from 258 apparently healthy individuals (129 females and 129 males) in the age range of 21-74. The reference range interval was calculated using non-parametric statistics representing the central 95% of the population. Results indicated a reference range for females is from 17.6 ng/mL to 159.3 ng/mL and that for males is from 19.4 ng/mL to 246.3 ng/mL. However, it is recommended that each laboratory establishes a range of normal values for the population it serves.

Results
Results are printed out in ng/mL. Note: Samples with values greater than 1000 ng/mL should be diluted with saline in 1:1 ratio and rerun. Multiply results by the dilution factor of 2. Samples with value below 13 ng/mL should be reported as < 13 ng/mL.

Limitations
As with any latex turbidimetric assays, Diazyme Ferritin Assay runs should be followed with appropriate and thorough wash steps. Please consult specific instrument manuals for further information.

Diazyme Ferritin Assay REAGENT, CALIBRATOR, and CONTROL should be stored at 2-8°C. **DO NOT FREEZE**.

Performance Characteristics
Representative performance data on the Hitachi 917 analyzer are given below. Result obtained in individual laboratories may differ.

Precision
The precision of the Diazyme Ferritin Assay was evaluated according to CLSI EP5-A2 guideline. In the study, 2 levels of serum based controls containing 112.8 and 318.2 ng/mL of ferritin, and four serum sample containing approximately 35.8, 247.7, 616.2 and 855.2 ng/mL of ferritin, respectively, were tested with 2 runs per day in duplicates over 20 working days. Results were calculated using the EP Evaluator software precision statistic template and summarized in the following table:

Sample	Mean	Within-Run %CV	Between-Run %CV	Between-day %CV	Total %CV
Control 1	112.8	1.2	1.2	5.0	5.3
Control 2	318.2	1.7	0	3.1	3.5
Serum 1	35.8	4.1	4.9	7.7	9.5
Serum 2	247.7	1.3	0	2.8	3.1
Serum 3	616.2	1.4	1.0	3.6	4.0
Serum 4	855.2	1.2	0	2.0	2.3

Two very low serum samples containing ferritin concentrations of 15.7 ng/mL and 23.4 ng/mL ferritin were tested with 2 runs per day in duplicates over 20 working days on Hitachi 917 according to CLSI EP5-A2 guideline. Results were calculated using the EP Evaluator software precision statistic template and summarized in the following table:

Sample	Mean	Within-Run %CV	Between-Run %CV	Between-day %CV	Total %CV
Serum 5	15.72	7.2%	4.2%	3.7%	9.1%
Serum 6	23.86	4.2%	3.1%	1.5%	5.5%

Lot-to-lot and instrument-to-instrument reproducibility studies were performed using three lots of the Ferritin reagent on three different Hitachi 917 analyzers. In this study, four human serum samples with 16.2 ng/mL, 32.3 ng/mL, 279.1 ng/mL, and 613.2 ng/mL ferritin were tested with 2 runs per day in duplicates over 20 working days. Results were calculated using the EP Evaluator software precision statistic template and summarized in the following table:

Sample	Mean	Within-Run %CV	Between-Run %CV	Between-day %CV	Between lot or inst CV%	Total %CV
Serum 7	16.2	5.5	4.2	4.7	2.8	8.4
Serum 8	32.3	4.4	3.5	0.0	0.9	5.6
Serum 9	279.1	2.3	2.3	2.2	1.4	3.9
Serum 10	613.2	2.6	1.1	0.8	0.1	2.9

Method Comparison
Human serum samples were tested with the Diazyme Ferritin Assay and the obtained results were compared to the predicate method using CLSI EP9-A2. A total of 91 unaltered individual serum samples (42 male and 49 female). All samples were tested in singlet with both methods.

The results are summarized in the following table:

Parameter	Linear Regression	Deming Regression
n	91	91
Slope	0.992	0.996
95% CI	0.974 to 1.010	0.978 to 1.013
Intercept	-3.205	-4.162
95% CI	-9.373 to 2.962	-10.336 to 2.011
Corre Coefficient (R)	0.9964	0.9964
Sample range	15.7 to 918.6	15.7 to 918.6

LOB, LOD, and LOQ
The LOB, LOD, LOQ of the Diazyme Ferritin Assay was determined according to CLSI EP17-A. The LOB was determined to be 6.0 ng/mL; the LOD was determined to be 9.2 ng/mL; the LOQ was determined to be 13.0 ng/mL.

Linearity
The linearity of the Diazyme Ferritin Assay was evaluated according to CLSI EP6-A guideline. The 95% CI and slope, intercept, and correlation coefficient (R^2) for the Deming regression and linear regression analysis are summarized in the following table:

Parameter	Linear Regression	Deming Regression
Slope	1.007	1.008
95% CI	0.988 to 1.026	0.989 to 1.026
Intercept	-11.74	-11.90
95% CI	-23.30 to -0.17	-23.47 to -0.33
R^2	0.9994	0.9994
95% CI	0.9988 to 0.9999	0.9988 to 0.9999

The assay Analytical Measuring Range (AMR) is 13 -1000ng/mL.

Matrix Comparison
To evaluate anticoagulant effects, 38 paired Serum, K_2 EDTA plasma, Lithium Heparin plasma samples were tested with the Diazyme Ferritin Assay with one replicate per sample in each set. To ensure that the concentrations of ferritin were distributed across the reportable dynamic range, some samples spiked with stock solution of ferritin were included.

Serum vs EDTA:

Parameter	Linear Regression	Deming Regression
n	38	38
Slope	0.986	0.987
95% CI	0.975 to 0.997	0.976 to 0.997
Intercept	1.01	0.93
95% CI	-1.98 to 4.00	-2.06 to 3.92
Corre Coefficient (R)	0.9995	0.9995
Sample range	14.7 to 931.2	14.7 to 931.2

Serum vs Heparin Plasma:

Parameter	Linear Regression	Deming Regression
n	38	38
Slope	0.944	0.945
95% CI	0.933 to 0.955	0.934 to 0.955
Intercept	3.10	3.02
95% CI	0.13 to 6.07	0.06 to 5.99
Corre Coefficient (R)	0.9994	0.9994
Sample range	13.4 to 912.2	13.4 to 912.2

The results showed that there was no significant matrix effect between serum, K_2 EDTA plasma, and Li Heparin Plasma.

Interference

To determine the level of interference from the substances present in serum, the Diazyme Ferritin Assay was used to test three serum samples with "low", "medium" and "high" Ferritin concentrations spiked with various concentrations of substances following Clinical and Laboratory Standards Institute EP7-A2. The following substances do not interfere with this assay at the levels tested (less than 10% bias):

Interferent	Concentration
Triglyceride	1000 mg/dL
Ascorbic Acid	176 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Hemoglobin	500 mg/dL
Rheumatoid Factor	200 IU/mL
Heparin	3000 IU/L
N-acetylcysteine	17.6 mM
Acetylsalicylic Acid	2.78 mM
Ampicillin	152 µM
Dobesilate	33.3 µg/ml
Na2-Cefoxitin	1549 µM
Ibuprofen	2425 µM
Levodopa	1.3 mM
Methyldopa	71 µM
Metronidazole	701 µM
Rifampicin	78.1 µM
Theophylline	222 µM
Phenylbutazone	650 µM
Valproic Acid	3.5 mM
Deferasiron	1.0 mM
Methotrexate	2.0 mM
Prednisone	0.5 mM
Ferrous Sulfate	1.0 mM

Hook Effect

No high dose hook effect was observed up to 6,000 ng/mL Ferritin.

References

1. Aisen, P., and Listowsky, I. 1980. Iron transport and storage proteins. *Annual Review of Biochemistry.* 49, 357–393.
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3. Wu, Alan H. B. Tietz Clinical Guide to Laboratory Tests Fourth Edition Page 392-394
4. Fischbach FT, Dunning MB III, eds. (2009). Manual of Laboratory and Diagnostic Tests, 8th ed. Philadelphia: Lippincott Williams and Wilkins.
5. Charles Moore, Jr, Michelle Ormseth, Howard Fuchs, Causes and Significance of Markedly Elevated Serum Ferritin Levels in an Academic Medical Center J Clin Rheumatol. 2013;19(6):324-328.

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