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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 52 mL R2: 1 x 13 mL

* Calibrators and Controls sold separate

Intended Use
The Diazyme Ferritin Assay is for the quantitative determination of ferritin in serum and plasma samples.

Background[1-3]
Ferritin is an iron-containing protein with a molecular weight of approximately 450,000 daltons. It is found mainly in the human liver, spleen and bone marrow, where its function is to eliminate and store iron. Serum ferritin is correlated with the available iron store in body and is an indicator of certain pathological conditions such as liver diseases, rheumatoid arthritis inflammation or malignancies in healthy subjects or patients. Pregnant women, blood donors, hemodialysis patients, adolescents and children are groups particularly at risk.

Assay Principle
The Diazyme Ferritin Assay is based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between Ferritin in a sample and anti-Ferritin antibodies which have been sensitized 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. USA: For Research Use Only. Not for in vitro diagnostic use.
2. EU: IVD
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 run with each new lot of reagents.
5. Avoid ingestion and contact with skin and eyes.
6. 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).

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.

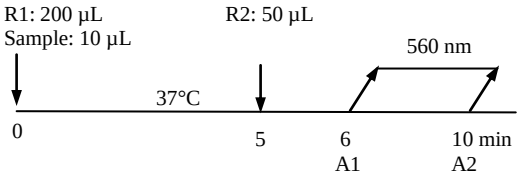
Specimen Collection and Handling
Serum, heparinized plasma, or K2 EDTA plasma samples can be tested with the Diazyme Ferritin Assay. For serum, collect whole blood by venipuncture and allow clotting. For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum 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 be centrifuged before assaying.

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

Test Procedure
Below is an assay test scheme for the Hitachi 917 analyzer.



The ferritin concentration in each sample is determined with the read absorbance change from a calibration curve prepared with calibrators of known concentrations. Application sheets for use of the Diazyme Ferritin Assay on other automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

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.

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 Labora-

tories (REF DZ526A-CON). Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results

Results are printed out in ng/mL. Note: Samples with values greater than 1000 ng/mL should be diluted with saline and rerun. Multiply results by the dilution factor.

Limitations

A sample with a ferritin level exceeding the linearity limit of 1000 ng/mL should be diluted with saline and reassayed incorporating the dilution factor in the calculation of the value. 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**.

Analytical 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-A guideline. In the study, 2 levels of serum based controls containing approximately 120 and 350 ng/mL of ferritin, and four serum sample containing approximately 35, 250, 600 and 850 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 tables:

Within-Run Precision

	Control Level 1	Control Level 2	Serum Level 1	Serum Level 2	Serum Level 3	Serum Level 4
N	80	80	80	80	80	80
Mean	112.8	318.2	35.8	247.9	616.2	855.2
SD	1.40	5.98	1.47	3.33	8.68	10.19
CV%	1.2%	5.3%	4.1%	1.3%	1.4%	1.2%

Total Precision

	Control Level 1	Control Level 2	Serum Level 1	Serum Level 2	Serum Level 3	Serum Level 4
N	80	80	80	80	80	80
Mean	112.8	318.2	35.8	247.9	616.2	855.2
SD	5.98	1.7	3.41	3.1	24.70	20.00
CV%	5.3%	3.4%	9.5%	7.7%	4.0%	2.3%

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 9.18 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 assay was linear from 13 to 1000 ng/mL.

Method Comparison

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 47 samples (ranging from 22.0 to 969.0 ng/mL of Ferritin) were tested in both assays in singlicate. Regular regression showed that the Diazyme Ferritin Assay results correlated well with a predicate method with a correlation coefficient (R^2) of 0.9873 with a slope of 1.0238 and y-intercept of -7.9175. Deming Regression Statistics revealed correlation coefficient (R) of 0.9936, slope of 1.031 (95% CI: 0.996 to 1.066), y intercept of -10.68 (95% CI: -25.36 to 4.68).

Matrix comparison

To evaluate anticoagulant effects, 40 paired Serum, K2 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. The results showed that there was no significant matrix effect between serum, K2 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 two serum samples with "low" 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	1500 IU/L
N-acetylcysteine	16.6 mM
Acetylsalicylic Acid	2.78 mM
Ampicillin	152 μM
Dobesilate	33.3 μg/ml
Na2-Cefoxitin	1549 μM
Ibuprofen	2425 μM
Levodopa	30.4 mM
Methyldopa	71 μM
Metronidazole	701 μM
Rifampicin	78.1 μM
Theophylline	222 μM
Phenylbutazone	650 μM
Valproic Acid	3.5 mM

References

1. Aisen, P., and Listowsky, I. 1980. Iron transport and storage proteins. *Annual Review of Biochemistry*. 49, 357–393.
2. Lipschitz, DA., Cook, JD. and Finche, CA. 1974. A clinical evaluation of serum Ferritin as an index of iron stores. *The New England Journal of Medicine*. 290:1213-1216
3. Wu, Alan H. B. Tietz Clinical Guide to Laboratory Tests Fourth Edition Page 392-394

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