



**TOTAL IRON REAGENT KIT**  
**V288-0B**

<b>Contents</b>	<b>Product No.</b>	<b>Package</b>
<b>TOTAL IRON KIT – B</b>	<b>V288-0B</b>	
Sample Diluent	V288-01	4 x 6 mL
Color Reagent (R2)	V288-02	1 x 7.8 mL
Ascorbic Acid	V288-03	4 x 60 mg

**REAGENT PREPARATION**

Prepare the Catachem Total Iron Working Reagent (R1) by pouring the entire contents of the Sample Diluent (V288-01) into the Ascorbic Acid (V288-03) vial. Mix gently until all of the material is dissolved.  
No preparation is needed for the Color Reagent (R2).

**REAGENT STORAGE AND STABILITY**

Store the unopened Sample Diluent (R1), the unopened Color Reagent (R2) and the unopened Ascorbic Acid at 15-30°C or at 2-8°C.

When stored as directed, the reagents are stable until the expiration date stated on the label.

Once the Working Reagent (R1) is prepared, it is stable for 7 days at 2-8°C

After opening, the Color Reagent (R2) is stable for 30 days at 2-8°C.

***NOT FOR USE IN UNPROFESSIONAL SETTINGS***

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TOTAL IRON REAGENT KIT
V288-0B
MANUAL/AUTOMATED APPLICATION

Intended Use

For IN VITRO quantitative determination of Total Iron in serum or plasma for automated and manual applications.

Clinical Significance

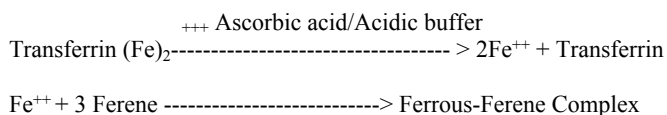
Measurements of Total Iron activity are primarily used for diagnosing hemolytic anemia, necrotic hepatitis, pernicious anemia, hemochromatosis, hemosiderosis, and lead poisoning, as well as for monitoring the causes and treatments.

Method History

Reports of direct determination of iron and iron-binding capacity were published by Schrade, et al in 1954. In 1970, Stookey used ferrozine, 3-(3-pyridyl)-5,6-bis(4-phenylsulfonic acid) 1,2, 4-triazine as a reagent for the colorimetric determination of iron, subsequently, a new, more sensitive ferrioxime-type reagent (5,5'-(3-(2-pyridyl)-1,2,4-triazene-5,6 diyl) bis-2-furansulfonic acid (ferene) became available. The major advantages of ferene are the high molar absorptivity (35,500), its water solubility, and stability over the pH range of 4 to 9. The Catachem Total Iron procedure is based upon the work of Stookey and the use of ferene.

Method Principle

In acid buffer, transferrin bound iron dissociates into free ferrous (Fe++) and ferric (Fe+++) iron. Ascorbic Acid present in the same acid reagent reduces the ferric iron to the ferrous state. The free ferrous ions react with ferene to form a complex with maximum absorbance at approximately 600nm. The absorbance is directly proportional to the concentration of Total Iron in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.



Reagent Content

When reconstituted according to the directions, the concentrations of the active ingredients will be approximately as follows:

Total Iron Diluent (R1)

Each liter contains:

- Buffer
Acetate 1 M
Thiourea 118 mM
Nonreactive ingredients and stabilizers.

Ascorbic Acid 10 mg/mL

Total Iron Color Reagent (R2)

Each liter contains:

- Ferene 6.0 mmol
Nonreactive ingredients and stabilizers.

Precautions

Catachem Total Iron reagents are for IN VITRO diagnostic use only. Handle these reagents following good clinical laboratory practice procedures. Avoid contact with skin and eyes. Should contact occur, wash affected areas with plenty of cold water. DO NOT PIPETTE REAGENTS BY MOUTH.

Reagent Storage And Stability

Store the Catachem Total Iron Reagent Kit at 2-8°C or 15°-30°C. When stored as directed, the reagents are stable until the expiration date stated on the label. The Catachem Total Iron Reagent Kit has been tested to reflect shipping conditions and is stable for the lifespan of the product if frozen up to 5 times or upon reaching temperatures up to 40°C for up to one week.

Preparation Of Working Reagent

Mix 10 ml Total Iron diluent with 100 mg of ascorbic acid to make R1 reagent. This is stable for 7 days after mixing. The Iron Color Reagent (Ferene R2 reagent) is used as is and is stable until the expiry date on the bottle label.

Reagent Deterioration

The reagent should not be used if signs of deterioration, such as cloudiness, are present. The reagents should be clear. If cloudiness is present, discard reagents.

Specimen Collection And Preparation

Plasma or serum specimens free of hemolysis should be collected. Sample specimens stored for periods longer than eight hours should be refrigerated at 2-8°C. Under these storage conditions, samples are stable for seven days. Handle specimens following good clinical laboratory procedures.

Quality Control

To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum

Interfering Substances

Specimens showing definite hemolysis are unsuitable for this procedure. Chelating agents such as EDTA should be avoided since it will erroneously lower the sample values. Dextran has been reported to interfere with iron procedures. All glassware and equipment used to prepare reagents and perform the test must be washed with 1 N HCl or 1 N nitric



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acid and rinsed thoroughly with plenty of distilled or deionized water <sup>(1)</sup>. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al <sup>(7)</sup>.

### Expected Values

The range of expected values determined for this method is 40 µg/dL to 150 µg/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located <sup>(1)</sup>.

### Procedure

Important: Read entire procedure instructions before proceeding with assay.

### Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1 ml adjustable for reagent
Pipette	0.100 ml for sample

### Materials Provided

Total Iron Diluent and Total Iron Color Reagent

### Calibration

Catachem calibrator, Catacal (C1200-10), is recommended for use in the iron assay. The calibrator and the unknown should be treated in the same way while performing the iron procedure.

### Analytical Parameters

Wavelength	600nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5.0 minute
Reagent Volume R1	200 uL
Reagent Volume R2	90 uL
Sample Volume	100 uL
Total Volume	390 uL
Sample to Reagent Ratio	1:3.9

### Assay Procedures

1. Pipette 200 uL of Total Iron Diluent Reagent R1 into each of three cuvettes marked, "Calibrator", "Sample," and "Blank."
2. Pipette 100 uL of Calibrator or Sample into their respective cuvettes. Use 100 uL of distilled water for the Blank. Mix all cuvettes well.
3. Set spectrophotometer wavelength at 600nm and zero the instrument with the Blank.
4. Incubate all cuvettes for 5.0 minutes at 37 degrees C.
5. Read the "Calibrator" and "Sample" absorbencies.
6. Pipette 90 uL R2 (Ferene color reagent) into each cuvette.

7. Incubate for 1 minute and read all absorbencies at 600nm.
6. Calculate the Total Iron concentration (µg/dL) in the sample(s), as shown in "Calculations and Results".

### Calculations And Results

$$T \text{ Iron } (\mu\text{g/DL}) = \frac{\text{Sample Absorbance (OD)}}{\text{Calibrator Absorbance (OD)}} \times \text{Calibrator } (\mu\text{g/dL})$$

Assay OD

Example:

Sample	0.08
Calibrator	0.09,
Calibrator	= 300 (µg/dL)

$$\text{Total Iron } (\mu\text{g/DL}) = \frac{0.08}{0.09} \times 300 \mu\text{g/dL}$$

$$= 267 (\mu\text{g/dL})$$

**Note:** Samples with iron concentrations greater than 1200 µg/dL should be diluted with physiological saline and reassayed. Results should be adjusted for dilution.

### Method Performance Characteristics

**Sensitivity:** The sensitivity of this method is 0.00010-0.00012 absorbance units per µg/dL.

**Linear Range:** In this method there is no significant nonlinearity over the range of 0-1200 µg/dL.

**Precision:** Within-run and day-to-day precision is summarized below.

### Precision Study

T IRON	Within-Run Precision		Total Precision	
	MEAN	SD	SD	CV
µg/DL	µg/dL	%	µg/dL	%
25	2.60	*	2.4	*
477	7.40	1.50	3.2	1.7
948	5.1	0.50	14.8	1.5

\*CV's not meaningful when the average approaches zero.

### Correlation

A comparison of this method using an automated analyzer was carried out against a reference method based on the Ferene reaction. The performance of Catachem Total Iron method was found essentially equivalent to the reference method used. Linear regression analysis produced the following information:

Range	=	7-245ug/dL
N	=	3
Y	=	0.989x - 2.0
r	=	0.961
Sy.x	=	9.9



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**References**

1. Fundamentals of Clinical Chemistry. Edited by N.W. Tietz, 1976. WB Saunders Co, Philadelphia.
2. Schrade A, Ogamo J, Reinhart R, Miller J. Bound iron and unsaturated iron binding capacity of serum Rapid and reliable quantitative determination. Proc Soc Exp Biol Med 87,442 (1954).
3. Stookey LL. Ferrozine: a spectrophotometric reagent for iron. Anal Chem 42 (7):779-781 (1970).

4. Artiss, MD Vinogradov S, Zak B. spectrophotometric study of several sensitive reagents for serum iron. Clin Biochem 14, 311-315 (1981).
5. Higgins T. Novel chromogen for serum iron determinations. Clin Chem 27, 1619 (1981).
6. Artiss JD, Strandbergh DR, Zak B. Study of continuous flow automation for serum iron on comparing several sensitive reagents. Microchemical Journal 28, 275-284 (1983).
7. Young DS, Pestaner LC, Gibberman V. Clin Chem 21, 203D (1975).
8. Ferene™ is a trademark of Sekisui Diagnostics