Technical Bulletin

Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Kit (Colorimetric)

Catalog Number MAK394

Product Description

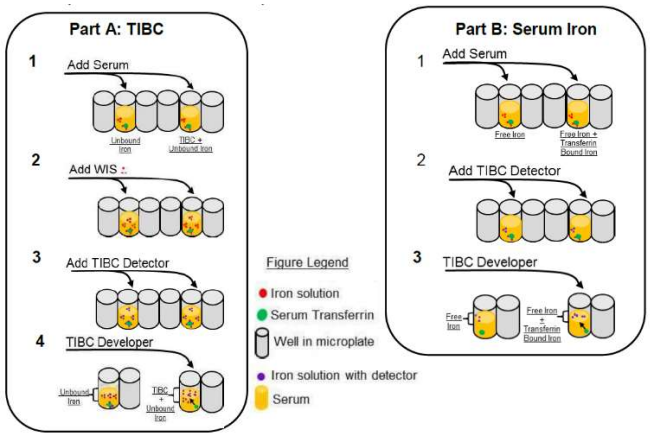
In humans, Transferrin is a blood protein that binds and transports iron throughout the body. Iron either bound or not bound to transferrin is reflected in the following:

- Total Iron Binding Capacity (TIBC)
- Unbound Iron
- Transferrin Saturation Bound Iron
- Free Iron

Measurements of these factors can be used to detect and monitor transferrin saturation, iron-deficiency anemia, and chronic inflammatory diseases.

The Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Kit measures both Total iron-binding capacity and Serum iron. Those values indicate the requisite iron for transferrin saturation and Serum Iron respectively.

The kit is suitable for the determination of TIBC, Unbound Iron, Transferrin Saturation Bound Iron, and Free Iron in serum or plasma.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- TIBC Assay Buffer 25 mL
  Catalog Number MAK394A
- Iron Solution 100 µL
  Catalog Number MAK394B
- TIBC Detector 2 × 1.5 mL
  Catalog Number MAK394C
- TIBC Developer 5 mL
  Catalog Number MAK394D
- Iron Standard (100 mM) 100 µL
  Catalog Number MAK394E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well flat-bottom plate. It is recommended to use clear plates for colorimetric assays. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader capable of temperature control at 25 ºC – 37 ºC

Precautions and Disclaimer

For Research Use Only. Not for uses in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 ºC, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

TIBC Assay Buffer: Bring to 37 ºC prior to use. Store at -20 ºC or 2-8 ºC.

TIBC Developer and Iron Standard: Store at -20 ºC or 2-8 ºC.

TIBC Detector: Store at -20 ºC, protected from light.

Procedure

Sample Preparation

Note: Off-the-clot serum is preferable to serum collected with an anti-coagulant. Use serum stored at -80 °C and avoid repeated freeze/thaw cycles.

For each sample, prepare duplicates for each (if needed):

- Unbound Iron
- TIBC + Unbound Iron
- Free Iron and Free iron + Transferrin Bound Iron

For TIBC Assay: Prepare two parallel wells for each sample dilution to determine Unbound Iron (A) and TIBC + Unbound Iron (B) (total of four wells including duplicates). Add 10-50 µL serum/well.

For Serum Iron Assay: Prepare two parallel wells for each sample dilution to determine Free Iron (C) and Free Iron + Transferrin Bound Iron (D) (total of four wells including duplicates). Add 10-50 µL serum/well.

Bring the total volume of each well to 50 µL with TIBC Assay Buffer.

Standard Curve Preparation

Note: The Standards can be prepared and added to the plate immediately prior to the final 10-minute incubation.

Prepare a 1 mM Iron Standard solution by diluting 10 µL of Iron Standard (100 mM) with 990 µL of purified water, mix well. Prepare Iron Standards according to Table 1. Mix well. Discard diluted 1 mM Iron Standard solution after use.

Table 1.
Preparation of Iron Standards

Well	1 mM Iron Standard	TIBC Assay Buffer	TIBC Detector	Iron (nmol/ well)
1	0 µL	225 µL	25 µL	0
2	2 µL	223 µL	25 µL	20
3	4 µL	221 µL	25 µL	40
4	6 µL	219 µL	25 µL	60
5	8 µL	217 µL	25 µL	80
6	10 µL	215 µL	25 µL	100

Working Iron Solution (WIS)

Immediately before use, prepare the Working Iron Solution (WIS) by adding 4 µL of Iron Solution to 996 µL of TIBC Assay Buffer. Make fresh solution as needed.

TIBC Assay

Add reagents as shown in Table 2 to Unbound Iron (A) and TIBC + Unbound Iron (B) wells in sequence. Incubate at 37 °C for time period specified at each step in Table 2.

Table 2.
TIBC Assay Reagents

Reagent	**Unbound Iron (A)**	**TIBC + Unbound Iron (B)**
WIS	125 µL	125 µL
Incubate for 10 minutes at 37 ºC		
TIBC Detector	25 µL	25 µL
Incubate for 10 minutes at 37 ºC		
TIBC Assay Buffer	50 µL	-
TIBC Developer	-	50 µL
Incubate for 10 minutes at 37 ºC		

Serum Iron Assay

Add reagents as shown in Table 3 to Free Iron (C) and Free Iron + Transferrin Bound Iron (D) wells in sequence. Incubate at 37 °C for time period specified at each step in Table 3.

Table 3.
Serum Iron Assay Reagents

Reagent	**Free Iron (C)**	**Free Iron + Transferrin Bound Iron (D)**
TIBC Assay Buffer	175 µL	125 µL
Incubate for 10 minutes at 37 ºC		
TIBC Detector	25 µL	25 µL
Incubate for 10 minutes at 37 ºC		
TIBC Developer	-	50 µL
Incubate for 10 minutes at 37 ºC		

Measurement

Measure absorbance at 570 nm (A_{570}) for Standards and all Samples. The A_{570} reading at the end of the final incubation is the value to be used in calculations. The plate may be measured between 24-37 ºC. However, each incubation should be performed at 37 ºC.

Results

1. Subtract 0 Standard reading from all Standards. Plot the Iron Standard Curve.
2. For each Sample, determine the absorbance due to TIBC ($A_{570\ TIBC}$) by using the following equation:

$$\mathbf{A_{570\ TIBC} = A_{570\ B} - A_{570\ A} =}$$

$$\mathbf{A_{570\ TIBC+Unbound\ iron} - A_{570\ Unbound\ Iron}}$$

3. For each Sample, determine the absorbance due to Serum Iron ($A_{570\ Serum\ Iron}$) by using the following equation:

$$\mathbf{A_{570\ Serum\ Iron} = A_{570\ D} - A_{570\ C} =}$$

$$\mathbf{A_{570\ Free\ Iron\ +Transferrin\ Bound\ Iron} - A_{570\ Free\ Iron}}$$

4. Apply the A_{570} values for TIBC ($A_{570\ TIBC}$) and Serum Iron ($A_{570\ Serum\ Iron}$) to the Iron Standard Curve to determine X and Y nmol, respectively, of iron in each Sample. TIBC and Serum Iron are represented as μmol iron/L (nmol/mL) of serum. Calculate the TIBC and Serum Iron as shown below:

$$\text{TIBC (μmol iron/L)} = \frac{X}{V} \times D \times 10^3$$

$$\text{Serum Iron (μmol iron/L)} = \frac{X}{V} \times D \times 10^3$$

$$\text{\% Transferrin Saturation} = \frac{\text{Serum Iron (μmol iron/L)}}{\text{TIBC (μmol iron/L)}} \times 100\%$$

where:

X = TIBC iron amount from Standard Curve (nmol)

Y = Serum Iron amount from the Standard Curve (nmol)

D = Sample dilution factor (if applicable; D = 1 for undiluted samples)

10^3 = Conversion factor mL to L

V = Volume of Serum Sample (μL)

Figure 1.
Typical Iron Standard Curve

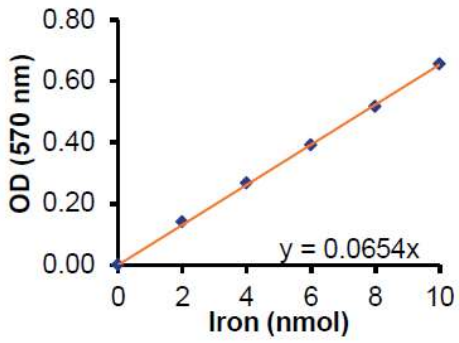
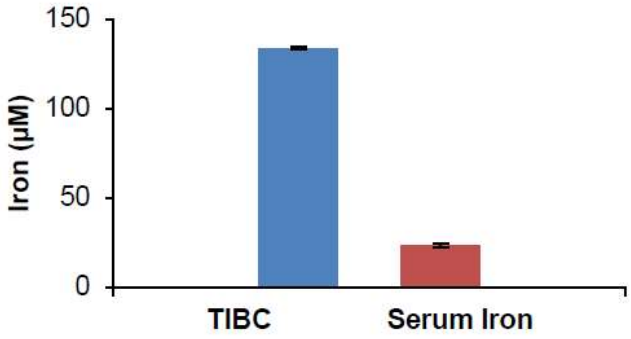


Figure 2.
Serum Iron and TIBC determination of Serum. Assays were performed following the kit protocol.



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