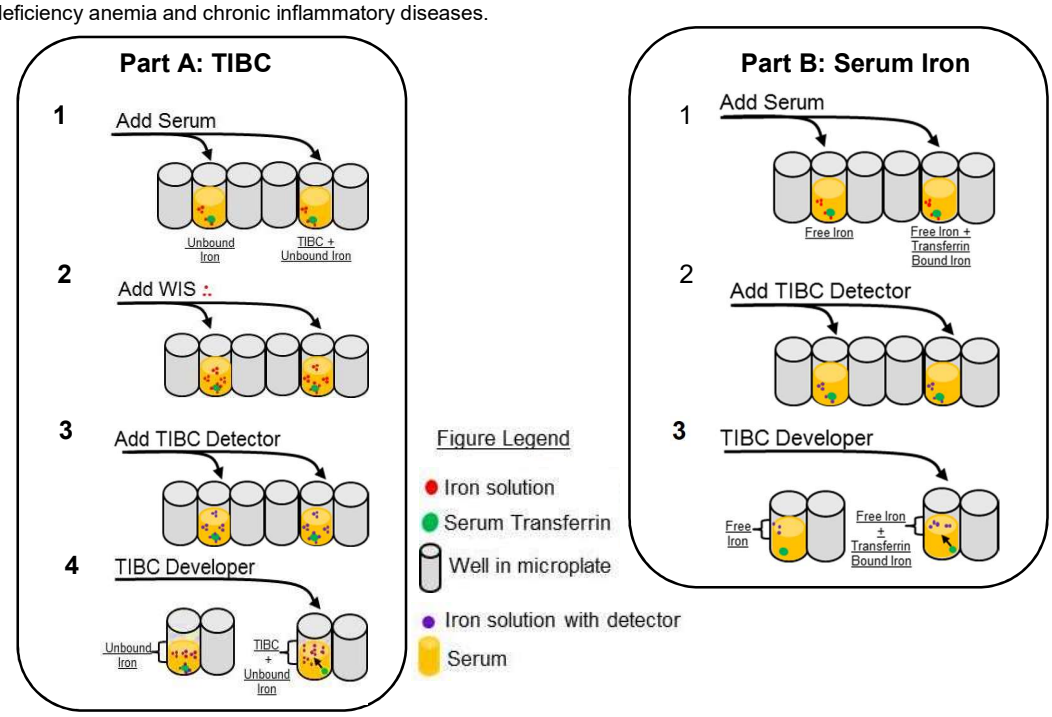
Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Kit (Colorimetric)

(Catalog # BN00650; 100 assays; Store at -20°C)

I. Introduction:

Assay Genie's TIBC and Serum Iron Assay Kit measures both Total iron-binding capacity (TIBC) and Serum iron. Those values indicate the requisite iron for transferrin saturation and Serum Iron respectively. In humans, Transferrin is a blood protein that binds and transports iron throughout the body. Iron bound to transferrin and not bound are reflected in the following: 1) Total Iron Binding Capacity, 2) Unbound Iron, 3) Transferrin Saturation Bound Iron, and 4) Free Iron. Those measurements can be used for to detect and monito transferrin saturation and also iron-deficiency anemia and chronic inflammatory diseases.



II. Application:

- Determination of TIBC, Unbound Iron, Transferrin Saturation, Serum Iron

III. Sample Type:

- Serum or plasma. Serum-off-the clot is preferable to normal serum.

IV. Kit Contents:

Components	BN00650	Cap Code	Part Number
TIBC Assay Buffer	25 ml	WM	BN00650-1
Iron Solution	100 µl	Blue	BN00650-2
TIBC Detector	2 x 1.5 ml	Brown	BN00650-3
TIBC Developer	5 ml	NM	BN00650-4
Iron Standard (100 mM)	100 µl	Yellow	BN00650-5

V. User Supplied Reagents and Equipment:

- 96-well plate clear plate with flat bottom
- Microplate reader capable of absorbance reading

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **TIBC Assay Buffer:** Bring to 37°C before use. Store at -20°C or 4°C.
- **Iron Solution:** Store at -20°C. Immediately before use, prepare the Working Iron Solution (WIS) by adding 4 µl iron solution to 996 µl TIBC Assay Buffer. Make fresh solution as needed.
- **TIBC Developer and Iron Standard:** Store at -20°C or 4°C. **TIBC Detector:** Store at -20°C. Keep protected from light.

VII. Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Protocol:

1. **Sample Preparation:** 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:** Wells 1-4 include two parallel wells for each sample dilution (Unbound Iron and TIBC + Unbound Iron). Add 10-50 µl serum/well. **For Serum Iron:** prepare two parallel wells for each sample dilution. Wells 5-8 include (Free Iron and Free Iron + Transferrin Bound Iron). Bring the final volume of each well to 50 µl with TIBC Assay Buffer.

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Notes:

a) Use serum stored at -80°C. Avoid repeated freeze/thaw.

b) Bilirubin concentrations up to 210 mg/L do not interfere with the assay.

2. **Iron Standard Curve:** Prepare 1 mM Standard: Add 10 µl of 100 mM Iron Standard + 990 µl dH_2O. Next, add 0, 2, 4, 6, 8, 10 µl of 1 mM Iron Standard to each well to generate 0, 2, 4, 6, 8 and 10 nmol/well Iron Standard. Bring to 75 µl final volume with TIBC Assay Buffer. Then, add 175 µl TIBC Assay Buffer followed by 25 µl TIBC Detector to each well. Discard diluted standard after use. *The standards can be prepared and added to the plate immediately prior to the final 10 minutes incubation.*

3. **TIBC & Serum Iron Assays:** Add reagents as specified in the tables below:

TIBC Assay		
	Unbound Iron (A)	**TIBC + Unbound Iron (B)**
WIS	125 µl	125 µl
Incubate @ 37 ºC for 10 minutes		
TIBC Detector	25 µl	25 µl
Incubate @ 37 ºC for 10 minutes		
TIBC Assay Buffer	50 µl	–
TIBC Developer	–	50 µl
Incubate @ 37 ºC for ten minutes		

Serum Iron		
	Free Iron (C)	**Free Iron + Transferrin Bound Iron (D)**
TIBC Dilution Buffer	175 µl	125 µl
Incubate @ 37 ºC for ten minutes		
TIBC Detector	25 µl	25 µl
Incubate @ 37 ºC for ten minutes		
TIBC Developer	–	50 µl
Incubate @ 37 ºC for ten minutes		

4. **Measurement:** Measure absorbance at OD: 570 nm for standards and samples. The OD at the end of the final incubation is the value to be used in calculations. The plate may be measured between 24°C-37°C. However, each incubation should be performed at 37ºC.

5. **Calculations:** Subtract 0 Standard reading from all Standards and plot the Iron Standard Curve. For each sample, determine the $TIBC_{(570\ nm)}$ by using the following equation: **$TIBC_{(570\ nm)}$= B – A** or $OD_{(TIBC+Unbound\ iron)} - OD_{(Unbound\ Iron)}$ **(See Step 3)**. Determine the Serum $Iron_{(570\ nm)}$ by using the following equation: **Serum $Iron_{(570\ nm)}$= D – C** or $OD_{(Free\ iron\ +\ transferrin\ bound\ iron)} - OD_{(Free\ iron)}$ **(See Step 3)**. Apply the OD values from $TIBC_{(570\ nm)}$ and Serum $Iron_{(570\ nm)}$ to the Standard Curve to get X and Y nmol respectively, of iron in each sample. TIBC and Serum Iron are represented as µmol iron/L of serum. Calculate the TIBC and Serum Iron as shown below:

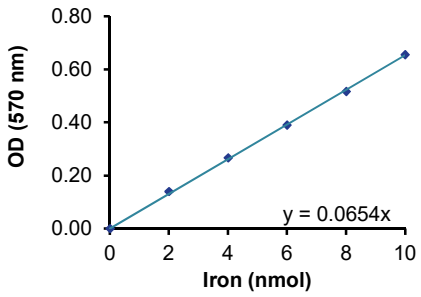
I) TIBC = $\frac{X}{V\ serum}$ **x dilution factor x 10^3 = µmol/L**

II) Serum Iron = $\frac{Y}{V\ serum}$ **x dilution factor x 10^3 = µmol/L**

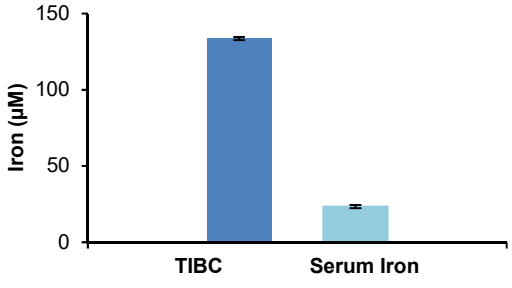
III) % Transferrin Saturation = $\frac{\text{Serum Iron}}{\text{TIBC}}$ **x 100**

Where: **X** is the TIBC iron amount from Standard Curve (nmol),
Y is the Serum iron amount from the Standard Curve (nmol),
10^3 is conversion factor mL → L,
V is the volume of serum sample (µl)

A)



B)



Figures: (A) Iron Standard Curve, **(B)** Serum Iron and TIBC determination of serum. Assays were performed following the kit protocol.

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