Version 5a Last updated 1 June 2023

ab239715 Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Kit

For the measurement of Total iron-binding capacity (TIBC) and Serum iron in serum or plasma.

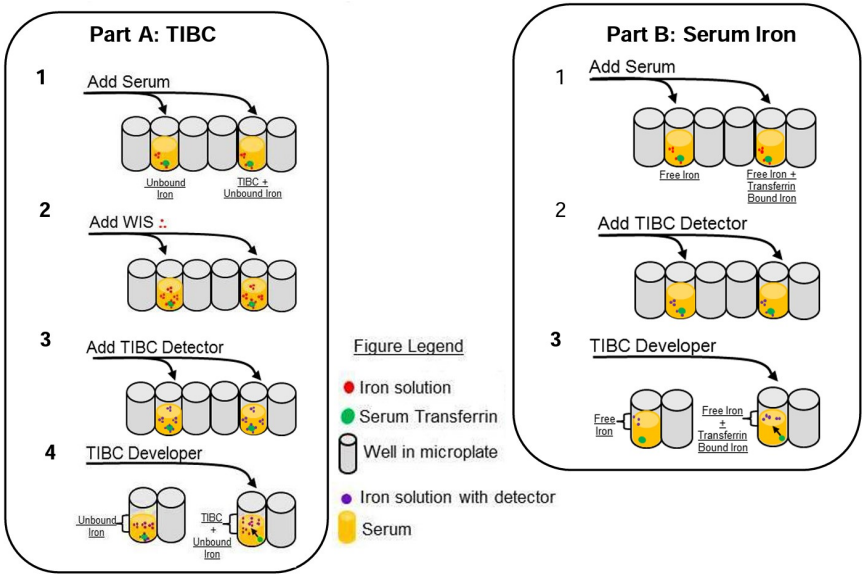
This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Total Iron-Binding Capacity (TIBC) and Serum Iron Assay Kit (ab239715) provides a simple, sensitive, and high-throughput adaptable approach to detect physiological Total iron-binding capacity (TIBC) and Serum iron. Those values indicate the requisite iron for transferrin saturation and Serum Iron respectively. Those measurements can be used to detect and monitor transferrin saturation and also iron-deficiency anemia and chronic inflammatory diseases.



2. Protocol Summary

Prepare samples, standards and controls.

For TIBC Assay, add WIS and incubate for 10 min at 37°C.

For Serum Iron Assay add TIBC Assay Buffer and incubate for 10 min at 37°C.

Add 25 µL TIBC Detector and incubate for 10 min at 37°C.

Add TIBC Developer Solution/TIBC Developer and incubate for 10 min at 37°C.

Measure absorbance at OD = 570 nm for standards and samples.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
TIBC Assay Buffer	25 mL	-20°C
Iron Solution	100 µL	-20°C
TIBC Detector	2 x 1.5 mL	-20°C
TIBC Developer Solution/TIBC Developer	5 mL	-20°C
Iron Standard II/Iron Standards (100 mM)	100 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom.
- Multi-well spectrophotometer.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 TIBC Assay Buffer:

Store at -20°C or 4°C. Bring to 37°C before use.

6.2 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.

6.3 TIBC Detector:

Store at -20°C. Keep protected from light.

6.4 TIBC Developer:

Store at -20°C or 4°C.

6.5 Iron Standard II/Iron Standard (100 mM):

Store at -20°C or 4°C.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare 1 mM Standard by adding 10 µL of 100 mM Iron Standard II/Iron Standard to 990 µL dH_2O.

7.2 Add 0, 2, 4, 6, 8, and 10 µL of 1 mM Iron Standard to each well to generate 0, 2, 4, 6, 8 and 10 nmol/well Iron Standard.

7.3 Adjust the volume to 50 µL/well with TIBC Assay Buffer.

Standard #	1 mM Iron Standard (µL)	TIBC Assay Buffer (µL)	Iron Standard/well
1	10	40	10 nmol
2	8	42	8 nmol
3	6	44	6 nmol
4	4	46	4 nmol
5	2	48	2 nmol
6	0	50	0 nmol

7.4 Add 175 µL TIBC Assay Buffer followed by 25 µL TIBC Detector to each well. Discard diluted standard after use.

ΔNote: The standards can be prepared and added to the plate immediately prior to the final incubation.

8. Sample Preparation

8.1 For each sample, prepare duplicates for: Unbound Iron, TIBC + Unbound Iron, Free Iron and Free iron + Transferrin Bound Iron.

8.2 For TIBC Assay: Include two parallel wells (Wells 1-4) for each sample dilution (Unbound Iron and TIBC + Unbound Iron). Add 10-50 µL serum/well. Bring the final volume of each well to 50 µL with TIBC Assay Buffer.

8.3 For Serum Iron: Prepare two parallel wells (Wells 5-8) for each sample dilution (Free Iron and Free Iron + Transferrin Bound Iron). Add 10-50 µL serum/well. Bring the final volume of each well to 50 µL with TIBC Assay Buffer.

Δ Note:

- Use serum stored at -80°C. Avoid repeated freeze/thaw.
- Bilirubin concentrations up to 210 mg/L do not interfere with the assay.
- This assay is not compatible with plasma samples collected with EDTA or citrate anticoagulants. Plasma samples collected in heparin are compatible.

9. Assay Procedure

9.1 Add reagents as specified in the tables below:

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

Serum Iron		
	Free Iron (C)	**Free Iron + Transferrin Bound Iron (D)**
Tibc Assay Buffer	175 µL	125 µL
Incubate at 37°C for 10 minutes		
TIBC Detector	25 µL	25 µL
Incubate at 37°C for 10 minutes		
TIBC Developer Solution/TIBC Developer	-	50 µL
Incubate at 37°C for 10 minutes		

9.2 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 37°C.

10. Data Analysis

10.1 Subtract 0 Standard reading from all Standard readings. Plot the Iron Standard Curve.

10.2 For each sample, determine the $TIBC_{(570\ nm)}$ by using the following equation:

$\mathbf{TIBC_{(570\ nm)}= B - A}$ or $OD_{(TIBC+Unbound\ iron)} - OD_{(Unbound\ Iron)}$

10.3 Determine the Serum $Iron_{(570\ nm)}$ by using the following equation:

$\mathbf{Serum\ Iron_{(570\ nm)} = D - C}$ or $OD_{(Free\ iron\ +\ transferrin\ bound\ iron)} - OD_{(Free\ iron)}$

10.4 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.

10.5 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 = $\frac{Serum\ Iron}{TIBC}$ x 100

Where: **X** is the TIBC iron amount from Standard Curve (nmol)
Y is the Serum iron amount from Standard Curve (nmol)
$\mathbf{10^3}$ is the conversion factor mL to L.
V is the volume of serum sample (µL)

11. Typical Data

Typical data provided **for demonstration purposes only**.

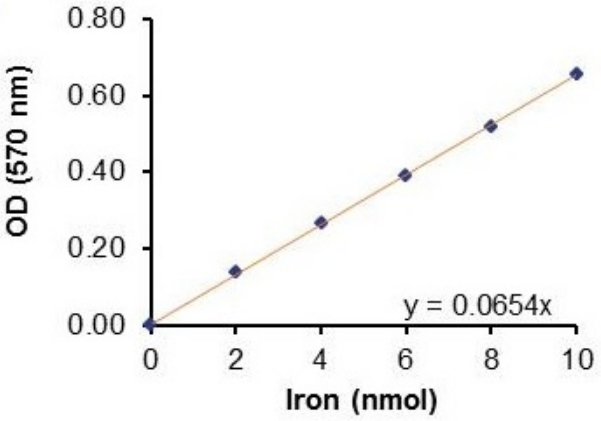


Figure 1. Iron Standard Curve.

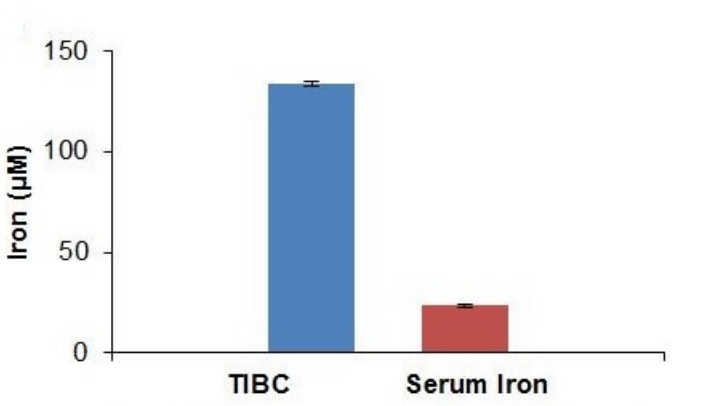


Figure 2. Serum Iron and TIBC determination of serum. Assays were performed following the kit protocol.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)