GENLISA™ Tissue Iron Assay (Colorimetric Method)

REF : KBCA2211

Ver 1.1

RUO

Quantitative Biochemcial Assay for the Determination of Tissue Iron in serum or plasma and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA2211  **50 tests**

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Introduction:

The GENLISA™ Biochemical Assays kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs an established biochemical method which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

The Tissue Iron Assay (Colorimetric Method) is a simple and sensitive technique for quantifying iron content in biological tissues. It involves forming a colored complex between Fe^{2+} and a chromogenic agent, with the color intensity being proportional to the iron concentration. This method is widely applicable to various tissue homogenates and is valuable for studying iron metabolism and related disorders.

Principle:

Under acidic conditions and the presence of a reducing agent, iron bound to transferrin is released from the protein, and Fe^{3+} in the serum is reduced to Fe^{2+}. The resulting Fe^{2+} then forms a pink-colored complex with dipyridine. Within a specific concentration range, the iron content exhibits a direct proportional relationship with absorbance, enabling quantitative analysis.

Materials Provided:

1. Iron Standard Stock Solution (100 mg/lt) - 1 x 1 ml
2. Iron Chromogenic Agent:
   Powder A – 1 vial
   Powder B – 1 vial
   Liquid C – 1 x 100 ml

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. All the reagents should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Reagent Preparation

Working Iron Standard Solution preparation (2mg/ml):
Take 0.2 ml of the iron standard stock solution and dilute it with distilled water to a final volume of 10 mL. Store the prepared solution at 4°C for future use.

Iron chromogenic agent:
Before use, add Powder A and Powder B into Liquid C and mix thoroughly until completely dissolved. The resulting iron chromogenic agent should be stored at 4°C, protected from light.

Assay Procedure:

1. **Sample pretreatment:**
   a. Animal tissue sample pretreatment: Accurately weigh the animal tissue sample and add physiological saline at a specified mass-to-volume ratio (e.g., 1 g of tissue per 9 ml of physiological saline).

Cut the tissue into small pieces and use a homogenizer or homogenate tube to prepare a 10% (w/v) tissue homogenate. Centrifuge the homogenate at 1,000–3,000 rpm for 10 minutes. Collect the supernatant, which serves as the 10% tissue homogenate, for further analysis.

b. Plant tissue sample pretreatment: Accurately weigh the plant tissue sample and add 0.1 mol/L phosphate buffer solutions (PBS, pH 7.0–7.4) at a specified mass-to-volume ratio (e.g., 1 g of tissue per 9 ml of PBS). Cut the tissue into small pieces and use a homogenizer or homogenate tube to prepare a 10% (w/v) plant tissue homogenate. Centrifuge the homogenate at 1,000–3,000 rpm for 10 minutes. Collect the supernatant, which serves as the 10% tissue homogenate, for further analysis.

Assay Protocol

	Blank	Standard	Sample
Distilled water (ml)	0		
(2mg/lt) Working Iron Standard Solution (ml)		0.5	
Blood Serum (ml)			0.5
Iron Chromogenic Agent (ml)	1	1.5	1.5
Mix thoroughly and place the sample tubes in a boiling water bath for 5 minutes (**blank and standard tubes do not require boiling**). Allow the tubes to cool to room temperature, then centrifuge at 3,500 rpm for 10 minutes. Carefully collect 1.0 ml of the supernatant and transfer it into cuvettes with a 0.5 cm light path. Measure the optical density (OD) of all tubes at 520 nm, using double-distilled water as the blank.for zero adjustment.			

Calculation:

Formula:

$$\text{Tissue iron content (mg/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (2mg/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

***** Iron content in standard tube is 2000 ug/L, iron's atomic weight is 55.847, so iron content in standard tube is 35.81 umol/L.

Example:

I. Take 0.5 ml of a 10% mouse liver homogenate to measure the iron content. The recorded optical density (OD) values are as follows: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.235. The protein concentration in the 10% mouse liver homogenate is 13.1365 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.237 - 0.002}{0.064 - 0.002} \times 35.81 \div 13.1365 = 10.3324 (\mu mol/\text{gprot})$$

II. Take 0.5 ml of a 10% mouse kidney homogenate to measure the iron content. The optical density (OD) values are: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.079. The protein concentration in the 10% mouse kidney homogenate is 10.5776 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81 μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.079 - 0.002}{0.064 - 0.002} \times 35.81 \div 10.5776 = 4.2045\ (\mu mol/gprot)$$

III. Take 0.5 ml of a 10% mouse cardiac muscle homogenate to measure the iron content. The optical density (OD) values are: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.078. The protein concentration in the 10% mouse cardiac muscle homogenate is 6.6536 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81 μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.078 - 0.002}{0.064 - 0.002} \times 35.81 \div 6.6536 = 6.5974\ (\mu mol/gprot)$$

IV. Take 0.5 ml of a 10% mouse lung tissue homogenate to measure the iron content. The optical density (OD) values are: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.094. The protein concentration in the 10% mouse lung tissue homogenate is 7.1655 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81 μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.094 - 0.002}{0.064 - 0.002} \times 35.81 \div 7.1655 = 7.4157\ (\mu mol/gprot)$$

V. Take 0.5 ml of a 10% mouse brain tissue homogenate to measure the iron content. The optical density (OD) values are: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.040. The protein concentration in the 10% mouse brain tissue homogenate is 4.4358 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81 μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.040 - 0.002}{0.064 - 0.002} \times 35.81 \div 4.4358 = 4.9479\ (\mu mol/gprot)$$

VI. Take 0.5 ml of a 10% mouse brain tissue homogenate to measure the iron content. The recorded optical density (OD) values are: OD_{Blank} = 0.002, $OD_{Standard}$ = 0.064, and OD_{Sample} = 0.053. The protein concentration in the 10% mouse brain tissue homogenate is 1.6378 g protein/L. The iron content is calculated as follows:

$$\text{Tissue iron content (μmol/gprot)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (35.81 μmol/L)} \div \text{Sample protein concentration (gprot/L)}$$

$$= \frac{0.053 - 0.002}{0.064 - 0.002} \times 35.81 \div 1.6378 = 17.9855\ (\mu mol/gprot)$$

Note:

1. Glassware must be thoroughly cleaned to prevent iron contamination. It is recommended to use disposable plastic test tubes to avoid any potential iron pollution.

2. If the supernatant appears turbid, transfer it to another test tube and centrifuge again to ensure clarity.
3. This method demonstrates strong analytical performance with minimal interference from other parameters, making it suitable for measuring iron content in various tissue homogenates.

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