FERRITIN

2 reagents ready for use without sample dilution

Reference : FNCOL-H00 : 2 x 50 ml R1 + 15 ml R2
Reference : FNCOL-B00 : 50 ml R1 + 5 ml R2

ü ***INTRODUCTION – CLINICAL BACKROUND***

Ferritin is a protein, which binds and stores iron. This is the major iron storage compound and it is synthesised by the liver. Serum ferritin is believed to reflect reliably the concentration of cellular ferritin. Pathological iron overload (hemochromatosis) may be either primary (i.e., idiopathic or familial) or secondary (acquired) as a consequence of alcoholism, thalassemia, or repeated transfusion. The diagnosis can be made by documenting elevation of serum ferritin levels in conjunction with increases in serum iron level. Iron deficiency is one of the most prevalent disorders of humans; assay of serum ferritin concentration is a high sensitive and reliable means of demonstration of this disorder.

ü ***SAMPLES AND REFERENCE VALUES***

Samples: serum; samples should be fresh.
Normal range for females: between 10 and 120 µg/l.
Normal range for males: between 20 and 250 µg/l.
Normal range for 6 months to 15 years children: between 7 and 140 µg/l.
Normal range for 1 month to 5 months children: between 50 and 600 µg/l.
Normal range for new-borns: between 25 and 200 µg/l.

ü ***PRINCIPLE OF TEST***

The human serum sample reacts upon colloidal gold coated with a mixture of monoclonal antibodies to ferritin. In the presence of ferritin, the particles agglutinate, which induces a red shift in the visible spectrum of the colloid. This induces an increase in optical density at 600 nm, which is directly proportional to the ferritin concentration in the sample.

ü ***REAGENTSTO BE USED FOR THE TEST .***

R1	**Buffer**	**FDBUF-050**	**2-8°C**
PEG buffer pH 8, detergents, sodium chloride, sodium azide (<1g/l) as preservative			

R2	**Ferritin Gold reagent**	**FNCOL-H15/B05**	**2-8°C**
mixture of monoclonal antibodies against human ferritin (± 20 µg/ml) coated on colloidal gold particles suspended in borate buffer, stabilisers and sodium azide (< 1g/L)			

cal	**6 calibrators**	**FNREK-000**	**2-8°C**
cal	**5 calibrators**	**FNRGK-000**	**2-8°C**
Human ferritin in synthetic biological fluid standardised from the ferritin international standard NIBSC code 94/572, sodium azide (< 1g/l).			

ü ***PRECAUTIONS***

For in vitro single diagnostic use. To be handled by entitled Personnel.
Products from human source were tested and found free from HBsAg and antibodies to HCV and HIV but this material should be treated just as carefully as potentially potentially infective.
Products containing sodium azide have to be handled with care; avoid ingestion and contact with skin and mucous membranes. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides.

ü ***PREPARATION AND REAGENTS STABILITY.***

The reagents are ready for use; once opened, they are stable until expiry date if stored stoppered in appropriate temperature conditions and without any contamination (avoid pipetting and decantation).

ü ***QUALITY CONTROL.***

Accuracy and reproducibility: analytical performances can be checked with the internal quality control serum of the laboratory or with the Liquichek™ (BIO-RAD) Control sera (see the values range obtained with DiAgam reagents and indicated on the accompanying BIO-RAD sheet).
Calibration: calibration curve and stability of calibration curve can be validated with the DiAgam calibration control (FNCON-002).
In case of analytical performances modification, calibrate the method again and contact the manufacturer if modifications are subsisting.

ü ***METHOD OF ANALYSIS AND CALCULATION.***

Opened analysers and photometers methodologies.
Guidelines and validated methodologies are available beside the manufacturer.

Detailed Procedure.
6 points calibration with the 6 calibrators kit (FNREK-000).
or 5 points calibration with the 5 calibrators kit (FNRGK-000).
Alternatively:
One point calibration, with a calibrator between 20 and 100 µg/l, in this case samples with levels above 300 µg/l should be reassayed after 10x dilution in physiological water.
The calibrator, the controls and unknown samples must not be diluted.
Wavelength: 600 nm.
Temperature: 37 °C.

Add successively, respecting sequence:

Blank reagent	**Calibrator**	**Sample**
20 µl $H_2O\varphi$	20 µl Calibrator	20 µl Serum or Plasma
+ 250 µl Buffer	+ 250 µl Buffer	+ 250 µl Buffer
Mix, allow to incubate 5 minutes at 37°C		
+ 50 µl Ferritin Gold	+ 50 µl Ferritin Gold	+ 50 µl Ferritin Gold
Mix at 37°C and record **within 30 seconds** the optical densities OD1 at 600nm.		
Allow to incubate 5 minutes at 37°C and record the optical densities OD2 at 600nm.		

1. **The final OD of calibrator =**
   (OD2 – OD1)calibrator – (OD2 – OD1)blank reagent
2. **The final OD of sample =**
   (OD2 – OD1)sample – (OD2 – OD1)blank reagent
3. **The ferritin concentration in serum/plasma =**
   final OD sample/ final OD calibrator x calibrator concentration

ü ***ANALYTICAL PERFORMANCES***

specificity		all antibodies are monoclonal
repeatability n=10		< 10% (> 10 µg/l)
reproducibility n=10		< 10% (> 10 µg/l)
analytical range		5.7 µg/l to 1000 µg/l
calibration stability		depending on the equipment > 4 weeks on analyser
interference :		
triglycerides		< 10% or 2 DS until 7.5 mmole/l
hemoglobin		< 10% or 2 DS until 200 µmole/l
bilirubin		< 10% or 2 DS until 500 µmole/l
heparin		< 10% or 2 DS until 0.5 g /l
sodium fluoride		< 10% or 2 DS until 2.4 g/l
EDTA		< 10% or 2 DS until 5 g/l
sodium citrate		< 10% or 2 DS until 5 g/l

ü ***BIBLIOGRAPHY***

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