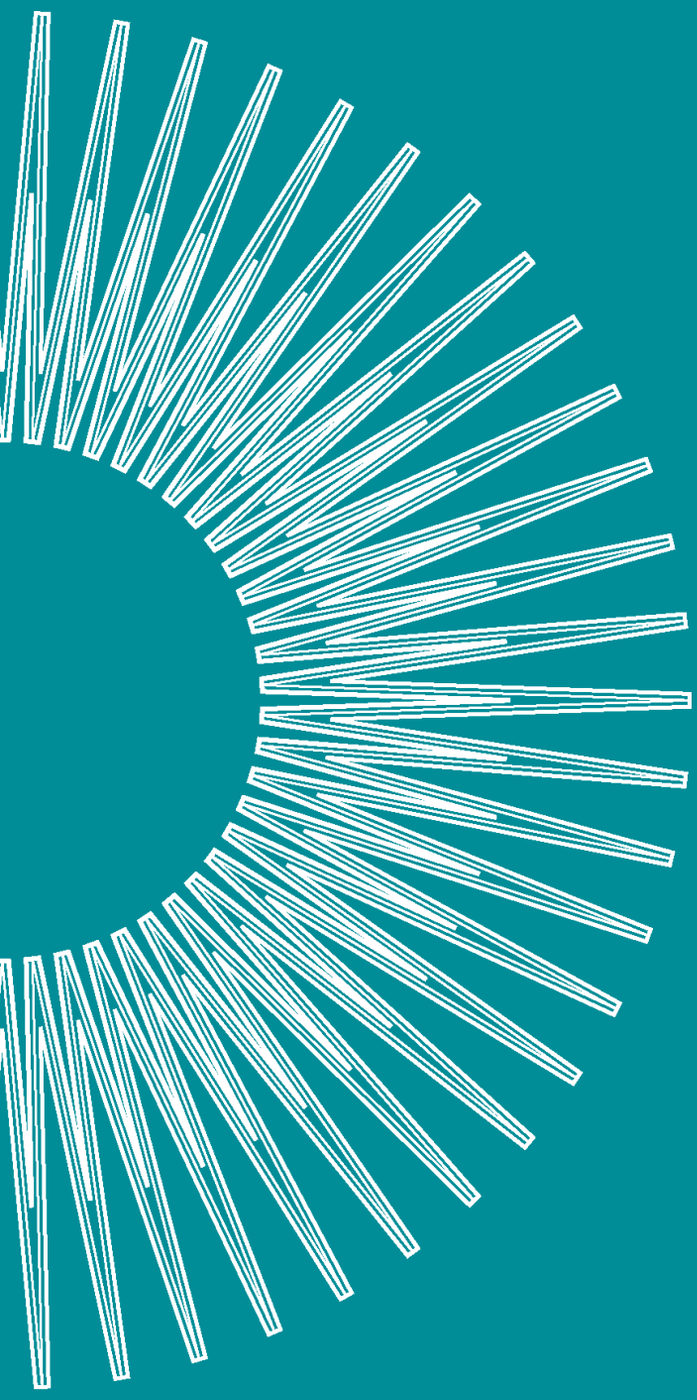
Performance Guide



iCheck
Iron
The test kit to measure iron in food

BioAnalyt

Performance **explained**

This Performance Guide is intended to explain the validity of our test kit iCheck Iron in a nutshell to facilitate your decision making.

Complex language and processes are used during performance evaluation of a test kit, referred to as validation.

To clarify and harmonize this terminology and processes we have hereby summarized how we validate our test kit and what the validation results mean.

We hope you find this material helpful and we are happy to receive your questions and comments! Do not hesitate to contact us at ***support@bioanalyt.com***.

Sincerely,
Your BioAnalyt Support team

TOP 100 INNOVATOR 2014

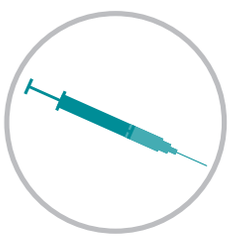
Development, manufacture and sales of all BioAnalyt test kits (devices, reagent vials) are carried out in accordance with ISO 9001:2008 and have been certified by TÜV NORD, Germany.

Note:
This material is based on the definitions set by ISO, the International Organization for Standardization in ISO 5725:1994.

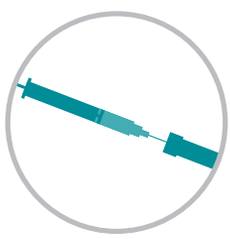
What is **iCheck Iron**?

iCheck Iron is an all-inclusive test kit for rapid on-the-spot measurement of iron in vitamin premix and food.

This test kit brings complex laboratory measurements down to a simple three-step process:



- Take up your sample

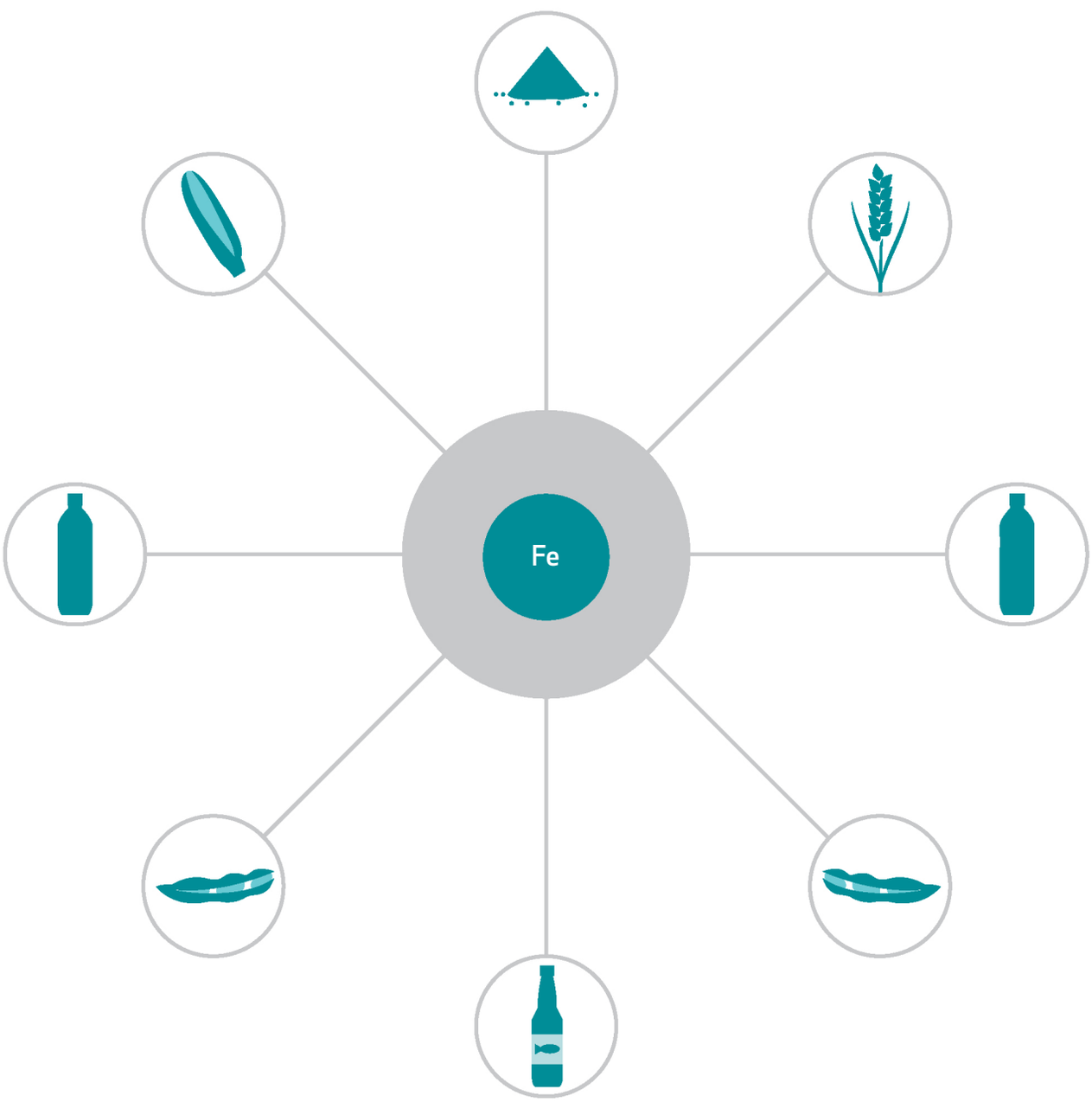


- Inject it into the ready-to-use reagent vial

- Measure the vial in your iCheck

What does **iCheck Iron** do?

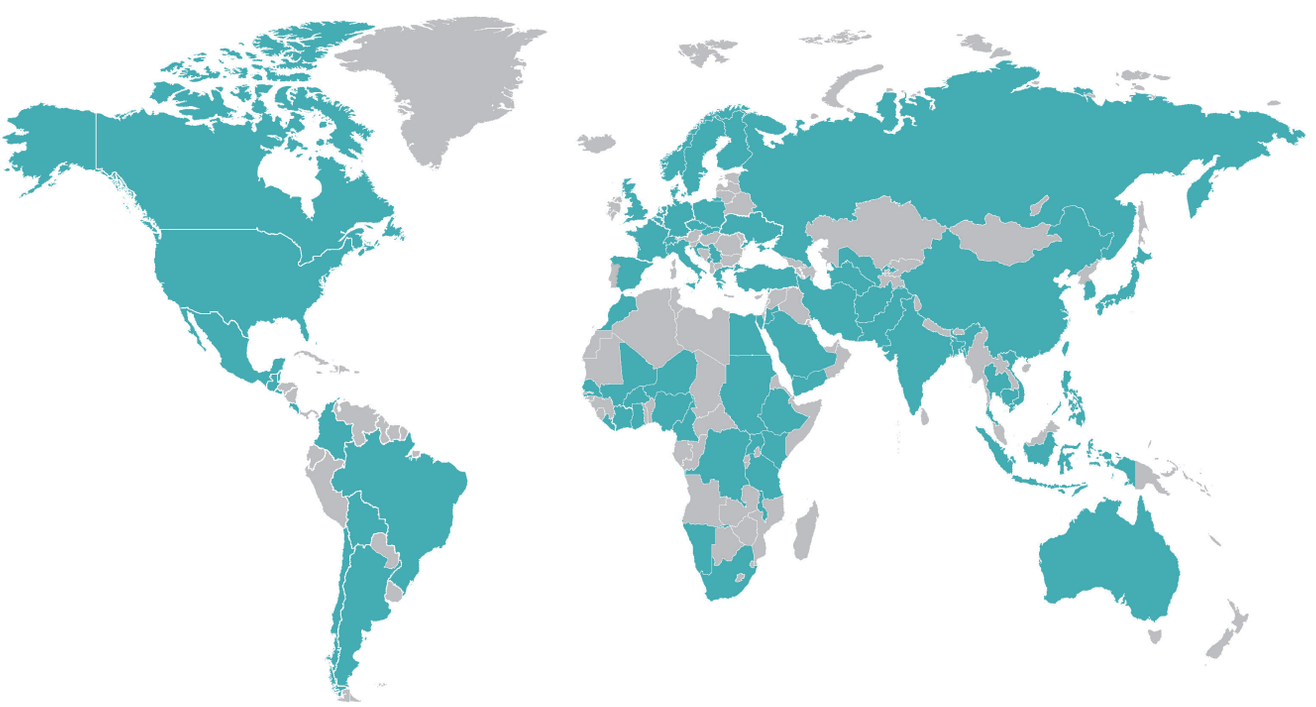
iCheck Iron measures iron as ferrous sulfate, ferrous fumarate and NaFeEDTA in vitamin premix, flour, beverages, soy and fish sauces.



Where are **iChecks** used?

iChecks are in use in over 80 countries around the globe.

Our customers are leading international organizations such as UNICEF, World Food Program, Hellen Keller International , Global Alliance for Improved Nutrition (GAIN), ministries and monitoring agencies, micronutrient premix producers, academic institutions, global and local food producers.

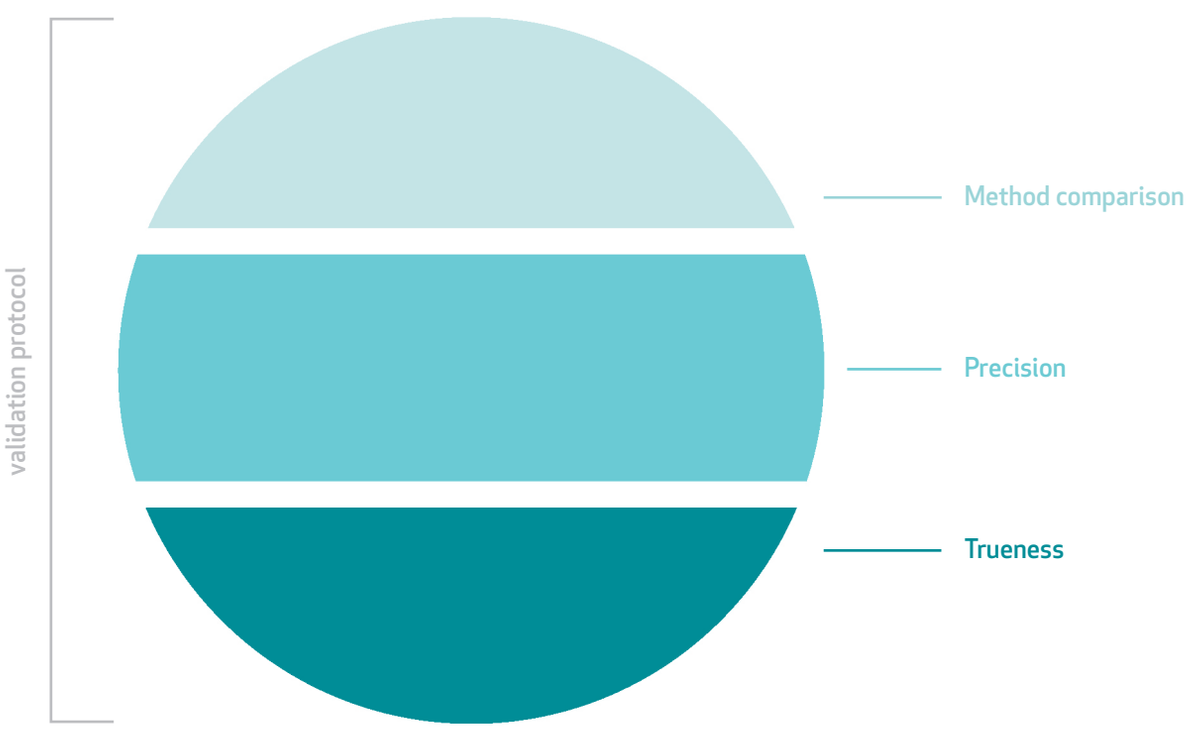


Countries using iCheck

Is **iCheck Iron** validated?

We assess the performance of each test kit following a rigorous standardized process. This process is called a validation protocol.

The validation protocol combines assessment of precision, trueness and a comparison to a reference method.



How is iCheck Iron validated?

1 Assessment of precision

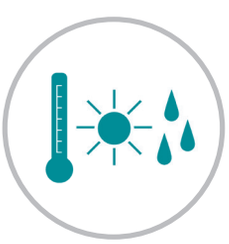
During the validation we assess the precision of the test kit. Precision tells us how similar are the repeated measurements of the same sample.

The difference observed between the repeated measurements is called variability of the result. The variability is common to all measurement methods and can be smaller or bigger.

Factors that affect the size of the variability of the result are:

- the sample itself

- the analyst



- the environment

- the instrument

We assess the precision by repeated measurements of the same sample under different conditions and by different people across the entire measurement range of iCheck Iron.

We have assessed the precision of iron measurement with iCheck Iron for the following sample types: iron standards, flour, fish and soy sauce.

The variability observed between the measurements is defined coefficient of variation (CV). CV is calculated by dividing the standard deviation with the mean of your repeated measurements. The CV for repeated measurements with iCheck Iron ranges between 3% and 16% depending on the type of food.

How is **iCheck Iron** validated?

2 Assessment of trueness

To know how close the measured result is to the real concentration of iron we assess the trueness of the measurement.

To do this we add a known concentration of iron to different types of samples and compare the expected concentration to the measured concentration.

The iron that we use is certified reference material which guarantees that our expected concentration is correct.

The trueness of iCheck Iron results is between 90% and 106% depending on the sample type. This means that of the expected 100% iCheck Iron measures 90-106% of the true iron concentration in the sample.

The difference between expected and measured result is called bias and for iCheck Iron results it is at 0 – 10%.

Certain samples, such as flour, fish and soy sauces contain natural iron, commonly called ***intrinsic iron***. In wheat flour the intrinsic iron content may vary between 10 mg/kg and 50 mg/kg. In fish and soy sauce the intrinsic iron content may vary between 0 mg/L and up to 100 mg/L.

iCheck Iron measures both intrinsic and added iron. Therefore to assess the concentration of added iron you need to deduct the intrinsic iron concentration from your result with iCheck Iron.

For more detailed information about the intrinsic iron and iCheck Iron measurement please contact as at ***support@bioanalyt.com***.

3 Method comparison

We further assess the performance of our test kit against an accepted reference method.

For iCheck Iron the reference method is AAS (Atomic Absorption Spectroscopy). AAS is a standard laboratory quantitative method for iron measurement in food as described in Codex Alimentarius issued by World Health Organization and Food & Agriculture Organization.

We measure in parallel the same samples with iCheck and with AAS and evaluate how the results agree with one another.

The method comparison shows us that 98% of iCheck Iron results are in agreement with AAS results. The results differ by a maximum of 70 mg/L and on average by as low as 2.3 mg/L*.

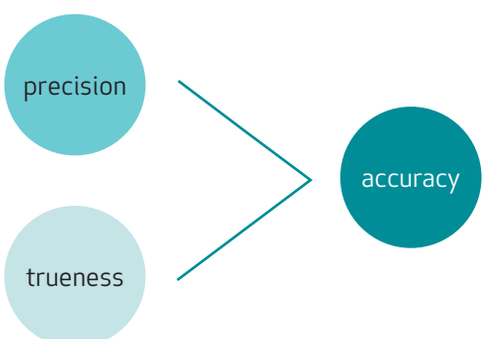
iCheck Iron AAS

Reference: Rapid quantification of iron content in fish sauce and soy sauce: A promising tool for monitoring fortification programs. A. Laillou, et al. Food and Nutrition Bulletin, vol. 34, no. 2 (supplement) , 2013.

How accurate are **iCheck Iron** results?

What is accuracy?
The assessment of the test kit's performance allows us to define how accurate iCheck Iron results are.

Accuracy combines both the precision and the trueness assessed during validation



How do we calculate accuracy?
We express the accuracy of iCheck Iron in terms of the uncertainty of measurement.

To calculate this uncertainty we take the coefficient of variation (CV) associated with precision and the bias associated with trueness and combine them using the following equation:

- Uncertainty = bias + 1.96 x CV

This equation gives us the uncertainty of measurement based on all our observations during the validation. The uncertainty of measurement gives a range to the result and a 95% confidence level that the true value lies within that range.

The uncertainty of measurement with iCheck Iron for different sample types is listed in the table below.

SAMPLE TYPE	IRON TYPE	TRUENESS	BIAS	CV	UNCERTAINTY OF MEASUREMENT
Iron standard	Ferrous sulfate	105%	5%	6%	**19%**
Iron standard	Ferrous fumarate	90%	10%	7%	**24%**
Iron standard	NaFeEDTA	101%	1%	3%	**7%**
Flour	Ferrous sulfate	100%	0%	8%	**16%**
Flour	Ferrous fumarate	98%	2%	16%	**34%**
Flour	NaFeEDTA	106%	6%	9%	**24%**
Fish and soy sauce	NaFeEDTA	103%	3%	7%	**17%**

Is this an acceptable accuracy?
It is important to note that the uncertainty does not imply doubt about the validity of a measurement. On the contrary, the knowledge of the uncertainty implies increased confidence in the validity of a measurement result.

The measurement of iron in different foods using the reference method AAS has the uncertainty between 5% and 30%, depending on the lab and the sample type Similarly, the measurement with iCheck Iron has uncertainty between 7% and 34%.

The result you obtain using iCheck Iron has an accuracy level which enables you to make a confident decision.

What does the uncertainty mean to you?
You have measured your flour sample that is fortified with NaFeEDTA and the result you have with the iCheck Iron is 45 mg/kg, after taking into account the dilution of solid sample in water.

The uncertainty of the iCheck Iron measurement is 24%. This mean that the true concentration of iron in your flour sample is in the range of 45 mg/kg ± 24%.

The result of the total iron in your flour sample is documented in the following way:

- 45 ± 11 mg/kg or
- 34 - 56 mg/kg.

If your flour sample has intrinsic iron content of 10 mg/kg, then the added iron is:

- 24 - 46 mg/kg.

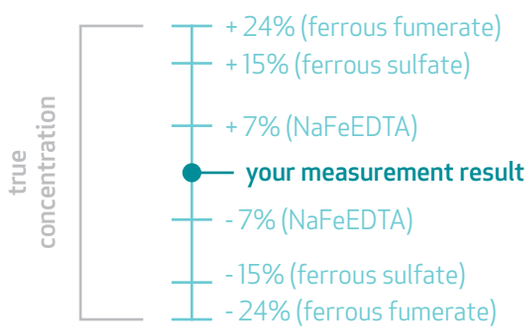
This range is then controlled against the required concentration.

How accurate are **iCheck Iron** results?

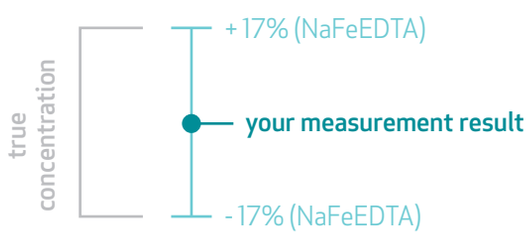
Accuracy and the sample type
The accuracy of your measurement depends to a large extent on the type of sample. Homogenous, in other words well-mixed samples such as iron standards, and sauces have lower measurement uncertainty of just 7-17%. On the other hand dry solid samples such as flour have lower homogeneity. This leads to higher measurement uncertainty of up to 34%.

Furthermore the accuracy also depends on the type of iron you measure. NaFeEDTA is soluble in water, can be mixed well and has higher homogeneity. This consequently leads to lower measurement uncertainty of just 7%. Ferrous fumarate, on the other hand, does not solubilize well in water and has lower homogeneity in water. This consequently leads to a higher measurement uncertainty of 24%.

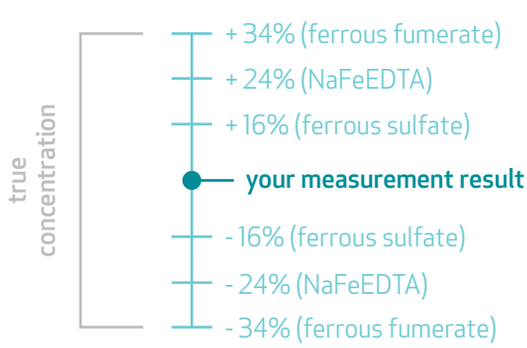
Iron Standard



Fish and soy sauce



Flour



Technical Data

The technical specifications for iCheck Iron are listed in the table to the right for your reference. If you would like more detailed information please contact us at ***support@bioanalyt.com.***

PARAMETER	DESCRIPTION
Analyte	Ferrous sulfate, ferrous fumerate, NaFeEDTA
Sample type	Vitamin premix, flour, soy and fish sauce, beverages
Sample preparation	None for liquid samples, homogenization and dilution in water for solids
Analysis method	Photometric
Units	mg/L
Linear range	1.5 – 12 mg/L
Time per measurement	from 5 minutes to 6 hours
Sample size	0.4 mL (400 µL)
Optimal temperature for measurement	20 – 30 °C
Uncertainty of measurement	3 – 30%
Reference method	AAS
Staff qualification	1 day training
Application	Laboratory and field
Energy source	Battery
Dimensions	11 x 4 x 20 cm (W x H x L)
Weight	0.45 kg

Glossary
of the terms used

AAS	*Atomic Absorption Spectroscopy. It is one of the standard laboratory quantitative method for iron measurement that involves atomization, irradiation and detection of the target analyte by the means of absorption.*
Accuracy	*closeness of an analytic result to an actual result. It is used to refer to both trueness and precision.*
Bias	*difference between expected and measured result due to systematic error of the measurement.*
Codex Alimentarius	*harmonized international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade. It was established and is maintained by Food & Agriculture Organization (FAO) and World Health Organization (WHO).*
CV	*Coefficient of Variation. It is calculated by dividing the standard deviation with the mean of your repeated measurements. CV = Standard deviation / Mean x 100%. CV is observed due to random errors of the measurement.*
Dilution	*mixing of dry solid sample with water to bring the solid sample into the liquid state for injection into the reagent vial. Dilution is also recommended with concentrated liquid samples to fit to the measurement range of iCheck Iron. The iCheck Iron measurement result obtained with the diluted sample must be multiplied with the dilution factor, in order to get the concentration value of iron in the solid or concentrated sample before dilution. The dilution factor is calculated by dividing the volume into which the sample was diluted with the weight of the solid or concentrated sample.*
Homogeneity	*uniformity of a substance or a uniform distribution of one substance within the other.*
ISO	*International Organization for Standardization*
Intrinsic iron	*natural iron present in organic samples. In wheat flour the intrinsic iron content may be between 10 mg/kg and 50 mg/kg. In fish and soy sauce the intrinsic iron content may be between 0 mg/L and up to 100 mg/L.*
Mean	*an average value of a set of values. It is calculated by summing up the values and dividing the sum with the number of values.*
Precision	*the extent to which a measurement procedure gives the same results each time it is repeated under identical conditions (repeatability) and variable conditions (reproducibility).*
Standard deviation	*a measure of the amount by which each value deviates from the mean of all values.*
Trueness	*closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value.*
Uncertainty of measurement	*the doubt that exists about the result of any measurement. It combines the random error (CV) and the systematic error (bias) following the equation: uncertainty = bias + 1.96 x CV. The uncertainty of measurement gives the result a range and there is 95% confidence that the true value lies within that range.*
Validation	*an analytical procedure performed with the objective to demonstrate that the analytical method is suitable for its intended purpose. During validation accuracy, precision, trueness, specificity and sensitivity of the analytical method for a certain analyte in a certain matrix is assessed.*
Variability	*a measure of the spread of a set of values from the reference or the mean value.*

Quality Guarantee

iCheck is produced following strict rules of quality assurance according to ISO 9001:2008. This is accomplished by the use of high-grade components and equipment as well as a stream-lined production process. This process includes quality controls of each component and rigorous calibration of the device by trained technicians.

Your iCheck Iron comes with a 2-year warranty.

If you have any questions, please contact us by calling ***+49 (0)33 28 35 15 000*** or sending an e-mail to ***support@bioanalyt.com.***

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This material has been developed with support from Global Alliance for Improved Nutrition.

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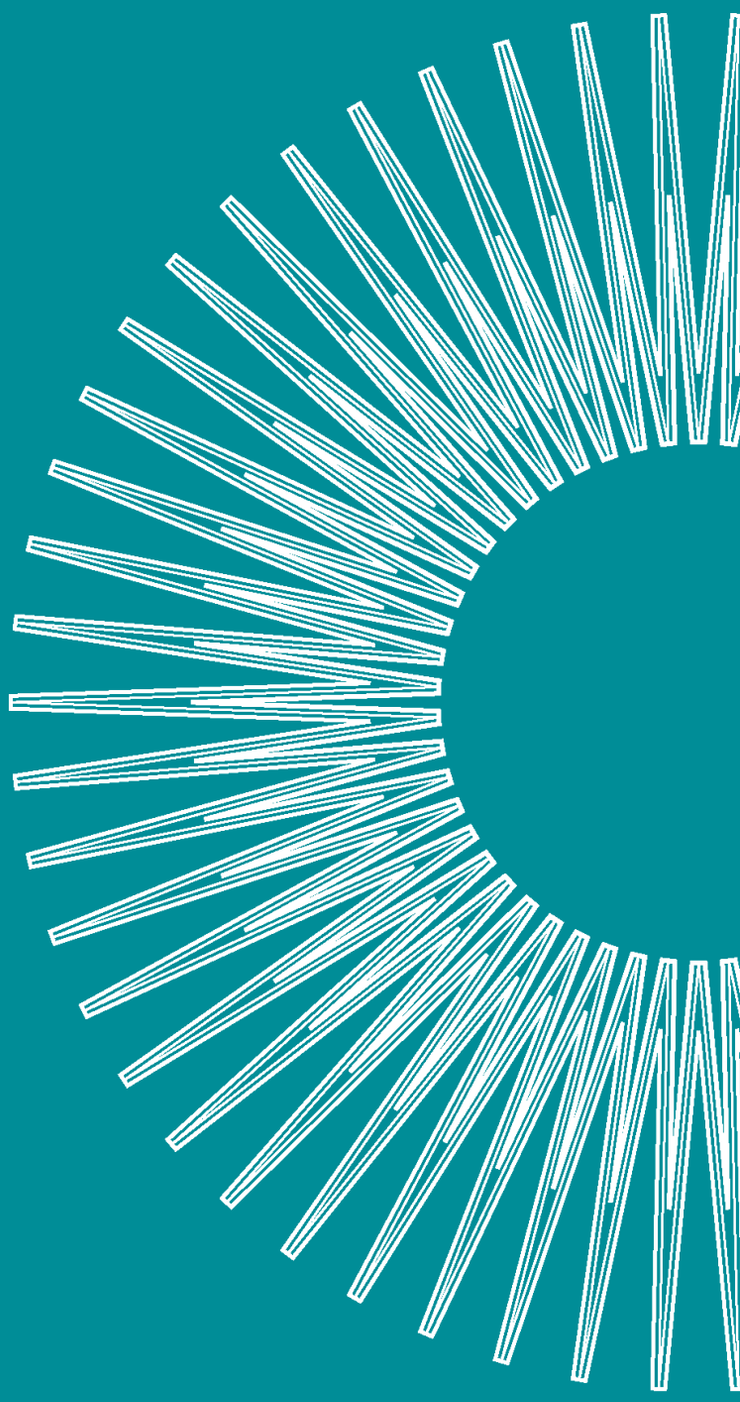

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