

iCheck Iron

Measuring Iron in Fortified Flour

1. Method Principle and Application

The [iCheck Iron device](#) is a single-wavelength portable photometer that converts absorption units into iron concentration in mg per liter (mg/L). The reagent vials supplied in the iCheck Iron Test Kit are developed to extract iron from water dispersible samples and detect iron using colorimetric reaction.



Iron quantification is based on a colorimetric reaction with bathophenanthroline. The resulting red complex correlates with iron concentration and is measured at 525 nm with the iCheck device. The measurement range of iCheck Iron is 1.5 – 12.0 mg/L.

The method is suitable for iron quantification in vitamin premixes, fortified rice kernels (FRK), flour, corn soya blend (CSB), lipid-based nutritional supplements (LNS), soy and fish sauce and beverages. Handling requires only one day training.

2. Iron in Fortified Flour

Iron can be added to flour in various forms which differ in their solubility. Therefore, it is necessary to identify the type of iron in the flour prior to sample preparation. This document details the dilution of fortified flour containing NaFeEDTA, ferrous fumarate or ferrous sulphate for subsequent iron content quantification with iCheck Iron.

Flour also contains natural (intrinsic) iron in the range from 10 mg/kg to 60 mg/kg and is also measured with iCheck Iron.

3. iCheck Iron Performance with Fortified Flour

iCheck Iron performance is assessed following a standardized process that combines assessment of precision, trueness and a comparison to a reference method (i.e. atomic absorption spectroscopy). The detailed description of this process is provided in the [iCheck Iron Performance Guide](#).

Performance of iCheck Iron with fortified flour containing different iron formulations has been assessed in internal validations. Below is a table detailing the observed precision and recovery.

Table 1. iCheck Iron Performance with Fortified Flour

Sample Type	Iron Type	Recovery	Coefficient of Variation
Wheat Flour	NaFeEDTA	106%	Max ±6%
	Ferrous Sulphate	100%	Max ±8%
	Ferrous Fumarate	100%	Max ±9%
Maize Flour	NaFeEDTA	103%	Max ±6%
	Ferrous Fumarate	98%	Max ±8%

4. Analyzing Added Iron Content in Flour Fortified with NaFeEDTA

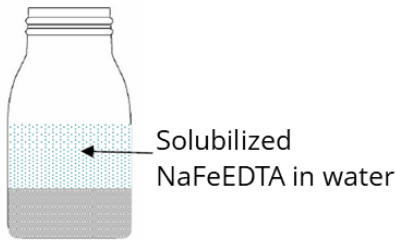
Ferric sodium ethylenediaminetetraacetate (NaFeEDTA) is a highly bioavailable compound easily solubilized in water. Follow the steps below to measure iron as NaFeEDTA with iCheck Iron.

- Weigh in your flour sample according to the expected iron concentration as shown in Table 2. Record the exact weight.

Table 2. Dilution of Fortified Flour for Iron Quantification with iCheck Iron

Sample Type	Expected Total Iron Conc. [mg/kg]	Dilution	Sample Weight [g]	Final Volume in Water [mL]	Diluted Sample Expected Conc. [mg/kg]
Wheat or Maize Flour	10 – 20	1 : 5	100	500	2 – 4
	20 – 60	1 : 10	50	500	2 – 6
	60 – 120	1 : 20	25	500	3 – 6

- Add approximately 100 mL of bottled or distilled water to a sample bottle, then add the weighed flour sample. Mix well.
- Fill up the sample bottle with water to a total volume of 500 mL. Record the exact final sample volume.
- Shake for minimum 5 minutes to solubilize NaFeEDTA.
- Let the solution stand and allow flour particles to sediment. Because NaFeEDTA is water-soluble, it will remain in the upper water layer.
- *Note: the intrinsic iron will sediment with the flour*



- To measure only added iron as NaFeEDTA, take up approximately 0.6 mL of the water layer with a syringe.
- Adjust the volume to exactly 0.4 mL and inject it into an activated iCheck Iron reagent vial. Shake vigorously for 10 seconds and let the vial stand for 1 hour, shake every 15 minutes during the hour.
- *Note: Maize flour may have thicker particles and sediment quickly – inject sample promptly! If particles do not fit through the syringe, grind the sample.*
- Proceed with measurement as described in the [iCheck Iron User Manual](#).

5. Analyzing Total Iron Content in Flour Fortified with Ferrous Fumarate or Ferrous Sulphate

Ferrous fumarate and ferrous sulphate are not readily soluble in water. Therefore, we recommend flour dilution in acid (0.2 – 0.4 M HCl) to ensure homogeneity of the diluted sample.

To prepare 0.2 M HCl:

- Dilute 17.5 mL of concentrated HCl (~36%) with 1000 mL of distilled water.
- HCl must be added to water, not the other way around.
- The solution is corrosive.
- The solution is enough for approximately 2 sample preparations and is stable for 6 months at room temperature.

This protocol is suitable for quantification of total iron (added + intrinsic) since most of the intrinsic iron will be solubilized in HCl along with added iron.

- Weigh in your flour sample according to the expected iron concentration as shown in Table 3. Record the exact weight.

Table 3. Dilution of Fortified Flour in HCl for Iron Quantification with iCheck Iron

Sample Type	Expected Total Iron Conc. [mg/kg]	Dilution	Sample Weight [g]	Final Volume in HCl [mL]	Diluted Sample Expected Conc. [mg/kg]
Wheat or Maize Flour	10 – 20	1 : 5	100	500	2 – 4
	20 – 60	1 : 10	50	500	2 – 6
	60 – 120	1 : 20	25	500	3 – 6

- Add approximately 100 mL of 0.2 – 0.4 M HCl to a sample bottle, then add the weighed flour sample. Mix well.
- Fill up the sample bottle with HCl to a total volume of 500 mL. Record the exact final sample volume.
- Shake the sample for a minimum of 5 minutes to homogenize the sample and obtain a slurry containing total iron.
- Shake the solution and immediately take up approximately 0.6 mL of the homogenized slurry into a syringe.
- Adjust the volume to exactly 0.4 mL and inject it into an activated iCheck Iron reagent vial. Shake **vigorously** for 10 seconds and let the vial stand for 1 hour, shaking it **vigorously** every 15 min.
- Proceed with measurement as described in the [iCheck Iron User Manual](#).



If there is a combination of NaFeEDTA and ferrous iron, or it is not known what type of iron is added, use the protocol with dilution in HCl to measure total iron in the sample.



6. Dilution Factor (DF) Calculation

The value displayed on the iCheck Iron after measurement will reflect the concentration of iron in the diluted sample. To obtain the original flour iron concentration, you must first calculate the dilution factor according to the following formula:

$$DF = \frac{\text{Total sample solution volume (mL)}}{\text{Sample weight (g)}}$$

Once you have calculated the dilution factor, multiply the iCheck Iron result by the dilution factor.

$$\text{Iron in Fortified Flour} \left(\frac{\text{mg}}{\text{kg}} \right) = \text{iCheck Iron result} \left(\frac{\text{mg}}{\text{L}} \right) \times DF$$

If you want to determine the amount of added iron after diluting in HCl, you must additionally correct for intrinsic iron:

$$\text{Added Iron Concentration} \left(\frac{\text{mg}}{\text{kg}} \right) = \text{Total Iron Result} \left(\frac{\text{mg}}{\text{kg}} \right) - \text{Intrinsic Iron} \left(\frac{\text{mg}}{\text{kg}} \right)$$

The intrinsic iron in flour concentration can range from 10 to 60 mg/kg, depending on the flour type, fine white flour has lower iron content, whole wheat flour would have high iron content due to higher bran content. The exact value can be obtained by measuring unfortified flour with iCheck Iron.

For technical support email us: support@bioanalyt.com

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