

iCheck Iron

Measuring Iron in Dry Vitamin Premix

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 soluble samples and detect iron using colorimetric reaction.



Acidic environment reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) which reacts with bathophenanthroline. The resulting red complex of iron and bathophenanthroline 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, flour, corn soya blend (CSB), lipid-based nutritional supplements (LNS), soy and fish sauce and beverages. Handling requires only one day training.

2. Working with Highly Concentrated Samples

If the expected iron concentration is outside of the iCheck device measurement range, the samples must be diluted, ideally to fit the middle of the measurement range. Highly concentrated samples, such as vitamin premix, should be handled with extra care due to a higher chance of error when working with low weights and small volumes.

Small errors lead to large variation!

Tips for accurate results

- Ensure your balance is well-calibrated.
- Record the exact amounts of samples weight and diluted volume for dilution factor calculation.
- Perform the dilution in multiple steps (serial dilution) if necessary.
- Ensure your diluted sample is homogenous.

4. Iron in Dry Premix

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

5. iCheck Iron Performance with Premix

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 premix containing different iron formulation has been assessed in internal validations. Below is a table detailing the observed precision and recovery.

Reference laboratory methods for iron analysis are AAS or ICP-OES. The recovery and method precision is primarily linked to the laboratory and not the method. The methods when executed correctly provide very high accuracy.

Table 1. iCheck Iron Performance with premix samples

| Sample Type | Iron Type | Recovery (dilutions to 5-12 mg/L solutions) | Coefficient of Variation |
|--------------------------------------|------------------|---|--------------------------|
| Vitamin Premix for wheat/maize flour | NaFeEDTA | 99±4% | Max. ±5% |
| | Ferrous Fumarate | 108±5% | Max. ±7% |
| | Ferrous Sulphate | 102±4% | Max. ±3% |



6. Analyzing Premix Containing Iron as NaFeEDTA or Ferrous Sulphate

Ferric sodium ethylenediaminetetraacetate (NaFeEDTA) is a highly bioavailable compound used as an iron fortificant. NaFeEDTA is water-soluble. Ferrous sulphate although is less bioavailable than NaFeEDTA is also soluble in water. Follow the instructions below to measure iron as NaFeEDTA or ferrous sulphate with iCheck Iron.

Table 2. Dilution of Premix containing NaFeEDTA or ferrous sulphate

| STEP 1 | Sample Type | Expected Concentration of iron [mg/kg] | STEP 1: Dilution | Premix Sample Weight [g] | Total Volume [mL] | Diluted premix concentration [mg/kg] |
|--------|--------------------|--|------------------|--------------------------|-------------------|--------------------------------------|
| | Dry Vitamin Premix | 10 000 - 20 000 | 1 : 100 | 1.00 | 100 | 100-200 |
| | | 30 000 - 90 000 | 1 : 100 | 1.00 | 100 | 300-900 |
| | | 100 000 - 150 000 | 1 : 100 | 1.00 | 100 | 1000-1500 |

| STEP 2 | DILUTED PREMIX CONCENTRATION FROM STEP 1 [mg/kg] | STEP 2: Dilution | Solution from STEP1 Weight [g] | Total Volume [mL] | Diluted premix concentration FINAL [mg/L] |
|--------|--|------------------|--------------------------------|-------------------|---|
| | 100-200 | 1 : 20 | 5.00 | 100 | 5-10 |
| | 300-900 | 1 : 100 | 10.00 | 1000 | 3-9 |
| | 1000-1500 | 1 : 200 | 5.00 | 1000 | 5-7.5 |

- **STEP 1:** Weigh in the premix according to Table 2 and STEP 1.
- Dilute premix to 100 mL with distilled or bottled water and shake until homogenized.
- *Note: Do NOT use refrigerated water. Water must be brought to room temperature.*
- Record the exact weight and total volume of your sample solution for dilution factor calculation.
- **STEP 2:** Take the diluted premix solution from STEP 1 and dilute again following the table "STEP 2".
- Record the exact weight and total volume of your final sample solution for resulting dilution factor calculation.
- Inject 0.4 mL of final premix dilution (from STEP 2) into activated Iron reagent vial, shake and let the sample stand at room temperature for at least 60 min, shaking it every 15 min.
- Proceed as described in the [iCheck Iron User Manual](#).



7. Analyzing Premix Containing Iron as Ferrous Fumarate

Ferrous fumarate is not readily soluble in water. Therefore, to ensure homogeneity, the sample should be diluted in acidic solution. Follow the instructions below to measure iron as ferrous fumarate with iCheck Iron.

Table 3. Dilution of Premix containing ferrous fumarate with 1M HCl

| STEP 1 | Sample Type | Expected Concentration of iron [mg/kg] | STEP 1: Dilution | Premix Weight [g] | Total Volume 1M HCl [mL] | Diluted premix expected iron conc. [mg/kg] |
|--------|--------------------|--|------------------|-------------------|--------------------------|--|
| | Dry Vitamin Premix | 10 000 - 20 000 | 1 : 100 | 0.5 | 50 | 100-200 |
| | | 30 000 - 90 000 | 1 : 100 | 0.5 | 50 | 300-900 |
| | | 100 000 - 150 000 | 1 : 100 | 0.5 | 50 | 1000-1500 |

2nd Dilution of HCl and Premix solution with water

| STEP 2 | DILUTED PREMIX IRON CONC. FROM STEP 1 [mg/kg] | STEP 2: Dilution | Solution from STEP 1 Weight [g] | Total Volume [mL] | FINAL Diluted premix iron conc. [mg/L] |
|--------|---|------------------|---------------------------------|-------------------|--|
| | 100-200 | 1 : 20 | 5.0 | 100 | 5-10 |
| | 300-900 | 1 : 100 | 10.0 | 1000 | 3-9 |
| | 1000-1500 | 1 : 200 | 5.0 | 1000 | 5-7.5 |

- **STEP 1:** Weigh in the premix according to Table 3 and STEP 1.
- Dilute premix to 50 mL with **1M HCl solution** and shake until homogenized. See next page for instructions how to prepare HCl solution
- *Note: Do NOT use refrigerated water. Water must be brought to room temperature.*
- Record the exact weight and total volume of your sample solution for dilution factor calculation.
- **STEP 2:** Take the acid diluted premix solution from STEP 1 and dilute again following the table "STEP 2". Use water in this step.
- Record the exact weight and total volume of your final sample solution for resulting dilution factor calculation.
- Inject 0.4 mL of final premix dilution (from STEP 2) into activated Iron reagent vial, shake and let the sample stand at room temperature for at least 60 min, shaking it every 15 min.
- Proceed as described in the [iCheck Iron User Manual](#).

8. Preparation of 1M HCl Solution

- Dilute 83 mL of concentrated HCl (~36%) with 417 mL of distilled water (HCl must be added to water, not the other way around).
- The solution is corrosive. Be cautious!
- The resulting volume of 500 mL of 1M HCl is enough for 10 sample preparations and is stable for 6 months at room temperature.

9. 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 premix iron concentration, you must first calculate the dilution factor according to the following formula:

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

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

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

For technical support email us: support@bioanalyt.com

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