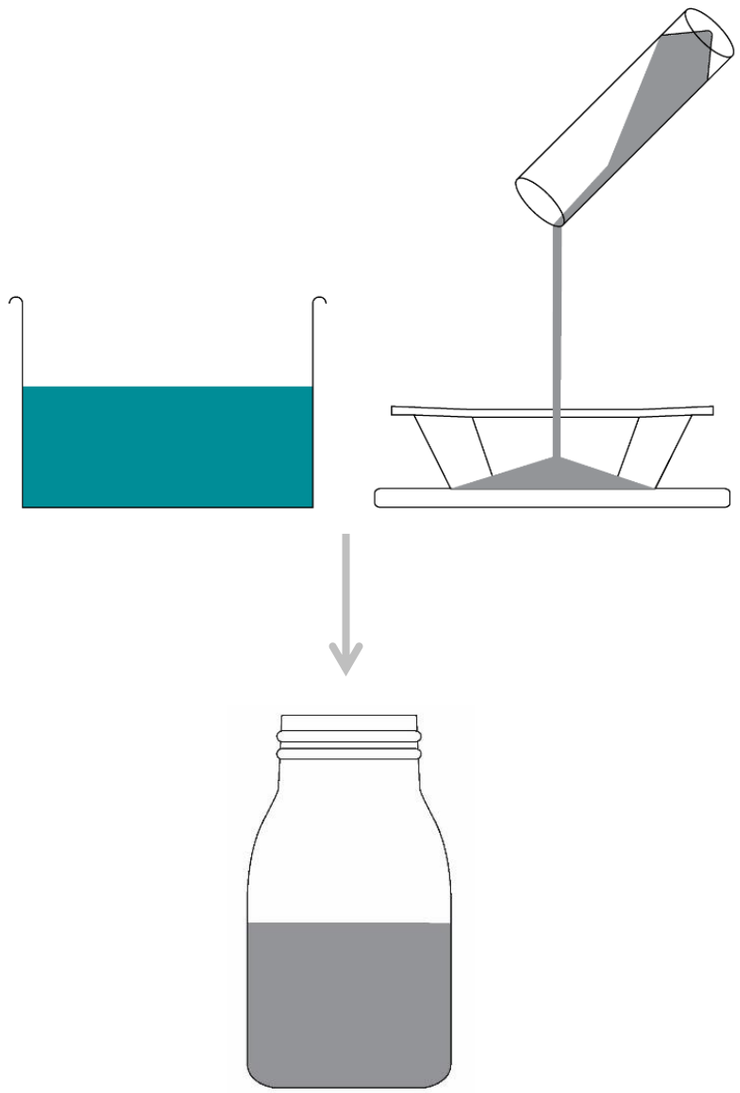
iCheck **Iron** :
FLOUR AND PREMIX sample preparation instructions

2019

Sample Preparation



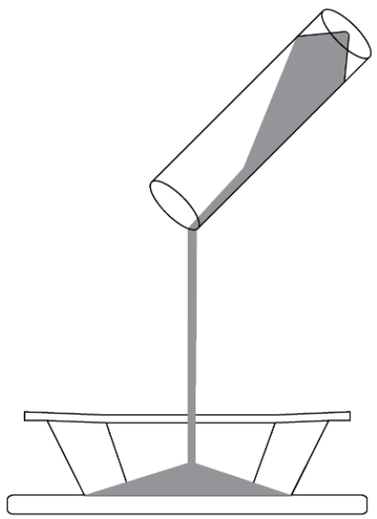
- Prepare the sample by diluting it with water.
- Sample preparation should be done if

1. expected concentration of your sample is above iCheck Iron measurement range (> 12.0 mg/L)
2. your sample is a solid sample.

Important!

- Iron concentration of the sample solution has to be in the measurement range of iCheck Iron which is 1.5 – 12.0 mg/L.

Weighing in the Sample



- Turn on the electronic balance provided with the iCheck case.
- Make sure your are in the mode to weigh in grams: “g”.
- Calibrate the balance using the weight provided with the iCheck case. Follow the instructions on the inside of the balance lid.
- Place weighing dish on the balance and press T/Z to tare the weight.
- Weigh in your sample and record

Calculations of Dilution Factor and Measured Concentration

1. **Dilution Factor (DF)** =
Total Diluted Sample Volume [mL] / Flour [g]

2. **Measured Total Iron [mg/kg]** =
iCheck Iron result [mg/L] x DF

3. **Measured Added Iron Concentration [mg/kg] =**
Total Iron Result [mg/kg] – Intrinsic* Iron [mg/kg]

***Contact support@bioanalyt.com to learn more about the intrinsic (natural) iron in different foods.**

Flour: Sample Preparation Step by Step

Natural Iron Content in Flour may range between 10 and 60 ppm. Make sure to consider that flour samples have intrinsic iron too in your calculations!

Sample Type	Expected Total Iron Conc. in Sample (mg /kg)	Dilution	Sample Weight (g)	Final Diluted Sample Volume (mL)
Wheat or Maize Flour	10 - 20	1 : 5	100	500
	20 – 60	1 : 10	50	500
	60 – 120	1 : 20	25	500

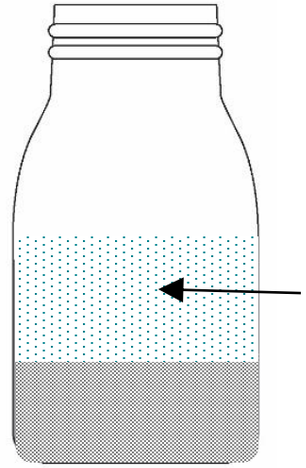
Attention

- Do not add the water to the sample. First add water, then sample.
- Do not use **refrigerated** water. It must be brought to room temperature.
- Do not use tap water. Use distilled or bottled water.

- Measure flour in grams according to the expected concentration (see table to the left). Record the exact weight.
- Dilute flour to a final volume of 500 mL with distilled or bottled water. Mix to get a homogenous slurry.
- *Tip to facilitate mixing: First add a portion of water (~ 100 mL) to the sample bottle, then flour. Mix well. Next fill up to 500 mL.*
- For dilution around 1:5 test if the sample solution can be taken in and out of the syringe with needle. Make sure the slurry is not too thick as it may block the needle.
- Record the total volume of your sample solution for dilution factor calculation.

Flour fortified with NaFeEDTA
Example

- Expected concentration of added iron in flour fortified with NaFeEDTA is 20 - 40 mg/kg (ppm).
- Dilute this flour with water; use 1:10 dilution. Take 50 g flour and mix it with 500 mL distilled or bottled water. Shake the diluted sample for min 5 minutes to solubilize NaFeEDTA.
- To measure **<u>added iron as NaFeEDTA</u>** let solution stand and flour particles to settle down. Take up into a syringe the upper water part of the solution. Inject into the iCheck Iron reagent vial and wait for 1 hour for reaction to complete and then measure.
- Multiply your reading with dilution factor. For water soluble iron dilution factor is calculated as follows: Water, ml/Flour, g = Dilution Factor.
- To measure **<u>total iron</u>** (added + intrinsic), follow the same instructions as for flour fortified with ferrous fumarate or sulphate.

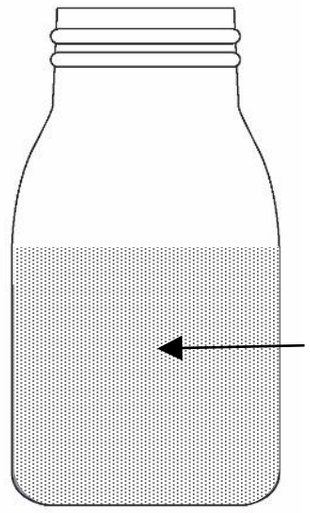


Water part with solubilized NaFeEDTA

Attention:
- **These instruction are only applicable to flour fortified with NaFeEDTA, NOT for ferrous fumarate, ferrous sulfate or electrolytic iron.**
- **Take note to new handling of Additive for activation of Reagent Vials (as of November 2018)**

Flour fortified with ferrous sulphate or fumarate: Example

- Expected concentration of added iron in flour fortified with iron fumarate is 20 - 40 mg/kg (ppm). Intrinsic iron content in flour varies between 10 and 60 ppm. Therefore the total iron concentration expected to be in this samples is 30 – 100 ppm.
- To dilute the distilled or bottled water may be used, however both ferrous sulphate and ferrous fumarate are poorly soluble in water. It is therefore recommended to dilute the flour in acid (i.e. **0.2-0.4M HCl**) to ensure homogeneity of diluted sample.
- Use 1:10 dilution. Take 50 g flour and add distilled or bottled water up to 500 mL. Shake the diluted sample for minimum 5 minutes to homogenize.
- Only **total iron** (added + intrinsic) can be measured in such samples.
- Shake the solution to get a homogenous slurry and immediately take the slurry into the syringe. Inject into the activated iCheck Iron reagent vial and wait for 1 hour for reaction to complete and then measure.
- Multiply your reading with dilution factor. For total iron dilution factor is calculated as follows: Final sample volume, ml/Flour, g = Dilution Factor.



Attention:
- **These instruction are NOT applicable for electrolytic iron.**
- **Take note to new handling of Additive for activation of Reagent Vials (as of November 2018)**

Vitamin Premix with NaFeEDTA

Sample Type	Expected Concentration, [mg/kg]	Dilution	Sample Weight	Volume of Water [mL]
Vitamin Premix	2 000 – 7 000	1 : 1000	1.00 gram	1000
	8 000 - 14 000	1 : 2000	0.50 gram	1000
	15 000 - 20 000	1 : 5000	0.20 gram	1000
	30 000 - 50 000	1 : 10 000	0.10 gram	1000
	60 000 - 140 000	1 : 20 000*	0.05 gram	1000

- Measure 0.01 g – 1.00 g of premix according to the expected concentration (see table to the left). Record the exact weight.
- Dilute premix to 1000 mL (1 L) with distilled or bottled water until homogenous solution.
- *Tip: Use analytical balance for weight below 0.1 g.*
- Record the total volume of your sample solution for dilution factor calculation.

Attention

- Do not use **refrigerated** water. It must be brought to room temperature.
- Do not use tap water. Use distilled or bottled water.

Vitamin Premix with Ferrous Sulphate or Ferrous Fumarate

Sample Type	Expected Concentration, [mg/kg]	Dilution	Sample Weight	Volume of Water [mL]
Vitamin Premix	2 000 – 7 000	1 : 1000	1.00 gram	1000
	8 000 - 14 000	1 : 2000	0.50 gram	1000
	15 000 - 20 000	1 : 5000	0.20 gram	1000
	30 000 - 50 000	1 : 10 000	0.10 gram	1000
	60 000 - 140 000	1 : 20 000*	0.05 gram	1000

- Measure 0.01 g – 1.00 g of premix according to the expected concentration (see table to the left). Record the exact weight.
- Ferrous fumarate is not readily soluble in water. Therefore to ensure homogeneity of diluted sample it is recommended to use acid instead of water.
- Dilute premix to in 100 mL of 0.2M HCl. Shake well for a minimum of 5 minutes. Then fill up the bottle with distilled or bottled water up to 1000 mL. Shake again.
- Record the total volume of your sample solution for dilution factor calculation.

Attention

- Use analytical balance for weight below 0.1 g.
- Do not use **refrigerated** water. It must be brought to room temperature.
- Do not use tap water. Use distilled or bottled water.

Example of Calculation for Premix

1. Expected Iron Concentration in Premix: 50 000 mg/kg (=ppm)

2. Weigh in 0.1 grams of premix and mix it to a final volume of 1000 mL water. Your dilution factor is: 1000 mL / 0.1 g = 10 000

3. Inject 0.4 mL of premix dilution into activated Iron reagent vial, shake and wait for the reaction.

4. iCheck Iron result is: 4.9 mg/L

5. Calculate the total iron concentration in the sauce: 4.9 mg/L x DF = 3.9 mg/L x 10 000 =
= 49 000 mg/kg

6. Add the uncertainty of measurement specific* for the food to your result.

   Measurement uncertainty for iron as NaFeEDTA in premix with iCheck Iron is: 7%.

   → 49 000 mg/kg * 7% = 3430 mg/kg

   → 49 000 ± 3430 mg/kg

   → <u>45 570 – 52 430 mg/kg</u>

***Read iCheck Iron Performance Guide to learn more about measurement uncertainty**

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