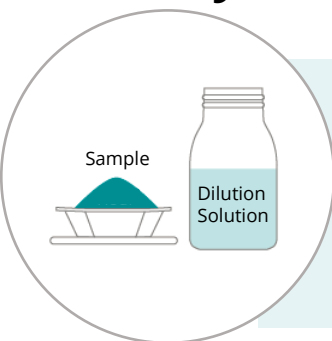


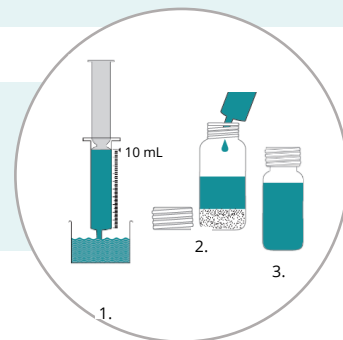
iCheck Iron

How to prepare your Fortified Rice Kernels (FRK) sample for analysis with iCheck Iron



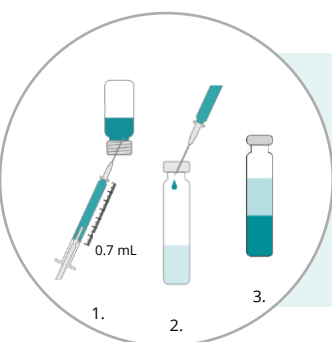
Step 1. Sample Preparation

- Consult the next page for detailed step by step preparation for FRK.
- The expected concentration in your diluted sample should be in the middle of iCheck Iron linear range [1.5 to 12.0 mg/L]. Based on the iron content in the FRK, the sample weight and volumes should be adapted (see table on page 4).
- Control your iCheck Iron device following the instructions in the iCheck Iron User Manual provided with your iCheck case.



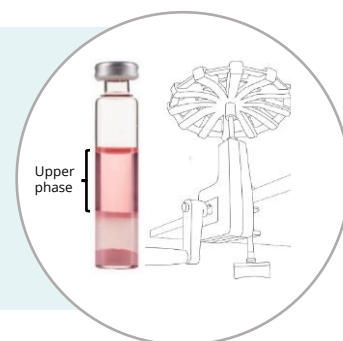
Step 2. Solubilize the ADDITIVE

- Add 10 mL of distilled or bottled water into the dry ADDITIVE vial with screw top. Use the big green 10 mL syringe provided in your test kit box (without a needle).
- Shake the vial until the ADDITIVE is completely solubilized.



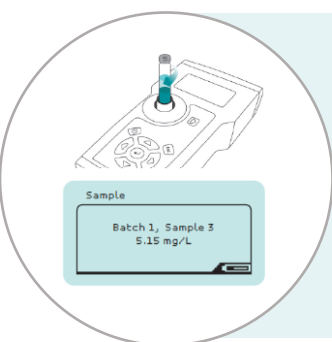
Step 3. Activate the Reagent vial with ADDITIVE

- Use small 1 mL syringe and thin green needle provided in your test kit box.
- Through red rubber septum take up 0.7 mL of solubilized ADDITIVE while holding the vial upside down. Make sure there are no air bubbles trapped inside the syringe.
- Inject 0.7 mL ADDITIVE into a reagent vial to activate it. Shake the vial for 10 seconds. Two clear phases are observed in the vial after adding the ADDITIVE.



Step 4. Reaction and Phase Separation

- Inject 0.4 mL of your diluted and homogenized sample into the activated reagent. Shake the vial vigorously for 10 seconds.
- Incubate the vial for 1 hour at 20-30 °C. Shake the vial every 15 minutes.
- At the end of the 1-hour incubation time, let the vial stand still until a clear phase separation appears. Centrifuge the vial using a manual hand centrifuge for not less than 30 seconds.



Step 5: Measurement and Calculation

- The vial is ready to be measured only if there is clear upper phase visible! Make sure that there are no particles stuck on the inside or outside of the vial in the middle.
- Measure the vial in your iCheck Iron following the instructions in your iCheck Iron User Manual.
- Multiply your result with your dilution factor (see page 4):
 - Iron in the sample [mg/kg] = iCheck Iron Result [mg/L] x DF

iCheck Iron

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Sample Preparation

1. Grind the kernels:

- Grind approx. 20 g FRK into fine flour-like powder using a coffee grinder.

2. Incubate FRK in NaOH:

- Transfer 25 mL of 0.6% NaOH solution* to a 50 mL falcon tube.
- Weigh 1.0 g of FRK powder and add it to 25 mL of NaOH solution.
- Shake immediately for 30 seconds.
- Incubate for 30 minutes at room temperature and shake for 30 seconds every 10 minutes.

3. Dilute the FRK sample fraction in HCl:

- Transfer 10 mL of 0.2 M HCl solution* to a 15 mL falcon tube.
- Invert the falcon with the FRK slurry to mix it and quickly take up approx. 0.8 mL into 1 mL syringe. Adjust to 0.5 mL.
- Add 0.5 mL FRK slurry to 10 mL of 0.2 M HCl solution.
- Shake the mixture for 10 seconds and let it stand for 5 minutes.

4. Sample Injection:

- Invert the diluted FRK sample before injecting it into activated iCheck Iron reagent vial.
- Shake the vial vigorously for 10 seconds and continue with the instructions on the first page under step 4.

* Details on how to prepare the solutions can be found on page 3

iCheck Iron

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How to prepare NaOH and HCl solutions:

0.6% NaOH:

- Dissolve 6 g NaOH in 1000 mL distilled water. Be aware that the NaOH has to be added to the water (not the other way around) and that the solution is corrosive.
- The prepared solution can be used for approx. 40 sample preparations and is stable for 6 months at room temperature when stored appropriately.

0.2 M HCl:

- Dilute 7 mL of concentrated HCl (~36%) with 400 mL of distilled water. Be aware that the HCl has to be added to the water (not the other way around) and that the solution is corrosive.
- The prepared solution can be used for approx. 40 sample preparations and is stable for 6 months at room temperature when stored appropriately.

Contact support@bioanalyt.com if you need further assistance regarding the dilution protocol.

iCheck Iron

Calculations

- iCheck Iron measurement range is 1.5 – 12.0 mg/L. If your sample solution is above or below this range, you need to adjust the dilution.
- Depending on the expected total iron concentration of the FRK, the sample weight and transfer volume of the FRK slurry must be adapted according to the table below.
- To calculate the measured iron, use the corresponding dilution factor and formula:

$$\text{Measured Iron [mg/kg]} = \text{iCheck Iron Result [mg/L]} \times \text{Dilution Factor}$$

	Expected Total Iron Concentration in FRK	Sample Weight	Volume of FRK slurry	Dilution Factor
A	700 – 1300 mg/kg	1.0 g	2 x 0.5 ml	275
B	1300 – 5500 mg/kg	1.0 g	1 x 0.5 mL	525
C	5500 – 10000 mg/kg	0.5 g	1 x 0.5 mL	1050

- Only use dilution procedure D in case the FRK slurry turns very viscous during the incubation in NaOH using dilution procedure B.

D	1300 – 5500 mg/kg	0.5 g	2 x 0.5 mL	550
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Attention

- The solubilized additive can be stored in the refrigerator at 4 °C and is stable for 6 weeks. The liquid might turn yellowish, this does not interfere with the test kit performance.
- Store reagent vials upright at room temperature (20-30 °C)