

iCheck Fluoro

Measuring Vitamin A in Flour

1. Method Principle and Application

iCheck Fluoro is a portable fluorometer for quantitative determination of the vitamin A content in vitamin premixes, sugar, flour and milk. The method is based on the fluorescence of the retinol molecule (excitation at 340 nm, emission at ≥ 400 nm). The device algorithm calculates vitamin A concentration in μg retinol equivalents (RE) per liter ($\mu\text{g RE/L}$). The measurement range of iCheck Fluoro is 50 – 3000 $\mu\text{g RE/L}$.



2. iCheck Fluoro Performance with Wheat and Maize Flour

iCheck Fluoro performance was assessed and compared to the reference method (i.e., high-performance liquid chromatography - HPLC) by Laillou and colleagues [1]. The table below summarizes the comparison of iCheck Fluoro results and to HPLC.

Table 1. iCheck Fluoro Performance compared to HPLC [1]

Sample Type	Dilution factor	Coefficient of variation iCheck Fluoro	Coefficient of variation HPLC	Correlation Coeff. HPLC vs. iCheck Fluoro (R^2)
Wheat flour T ₅₅	1:10	Max. $\pm 18\%$	Max. $\pm 30\%$	>0.91
Wheat flour T ₁₁₀	1:10	Max. $\pm 18\%$	Max. $\pm 10\%$	>0.91
Maize/Corn flour	1:10	Max. $\pm 8\%$	Max. $\pm 15\%$	~ 0.89

3. Vitamin A in Fortified Flour

The added vitamin A concentration in fortified flour is typically 0.5 – 3.0 mg RE/kg of flour. The most common form of vitamin A added to flour is retinyl palmitate. The recommended dilution for flour with such low added vitamin A levels is 1:5. At this dilution, the high concentration of the flour particles in the diluted samples leads to background fluorescence when analyzed with iCheck Fluoro. This causes the reading of vitamin A concentration to be overestimated.

To ensure accuracy, this background signal should be accounted for by measuring a non-fortified flour sample at 1:5 dilution and subtracting its reading from the test sample. Generally, the matrix effect value is 0.3-0.65 mg RE/kg for wheat flour and 0.1-0.4 mg RE/kg for maize flour.

4. Analyzing added Vitamin A in Wheat and Maize Flour

The measurement range of iCheck Fluoro is 50-3000 µg RE/L. The flour sample must be diluted in bottled or distilled water to fit the measurement range.

Weigh in the flour according to Table 2 and record the exact weight.

Table 2. Dilution of Flour for Vitamin A Quantification with iCheck Fluoro

Sample Type	Added Vit. A Conc. [mg/kg]	Added Vit.A Conc. [IU/kg]	Dilution	Flour Sample Weight [g]	Total diluted volume [mL]	Expected diluted flour conc. [µg RE/L]
Flour	0.5-3.0	1500-10000	1:5	100	500	100-600

- Transfer your sample to a 500mL or 1000 mL bottle and dilute it to a total volume of 500 mL. Shake until homogenized.
- 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.
- **Vitamin A is not stable in solution with water!** Proceed with measurement immediately.
- Vitamin A is not soluble but only dispersible in water. Therefore, if the diluted flour is standing still, vitamin A will separate from the water. Shake the solution and immediately take it up into the syringe.
- Inject 0.5 mL of the sample solution into a new iCheck Fluoro reagent vial. Shake the vial vigorously for 10 seconds. Proceed as described in the [iCheck Fluoro User Manual](#).

5. Dilution Factor (DF) Calculation

The value displayed on iCheck Fluoro after measurement will reflect the concentration of vitamin A in the diluted sample. To obtain the vitamin A concentration in dry flour sample, 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 Fluoro result by the dilution factor.

$$\text{Vitamin A in flour} \left(\frac{\text{mg RE}}{\text{kg}} \right) = \text{iCheck Fluoro reading} \left(\frac{\mu\text{g RE}}{\text{L}} \right) \times \frac{\text{DF}}{1000} - \text{reading with nonfortified sample (matrix effect)}$$

To correct for matrix effect, simply subtract the value of your reading with nonfortified sample measurement or the average matrix effect value for your sample type.

6. Vitamin A Unit Conversion

Below, you can find the relationship between retinol equivalents and other units used for vitamin A measurement, and for converting retinol palmitate/acetate to retinol.

- **1 mg Vitamin A = 1 mg retinol = 1 mg RE**
- **1 mg RE = 3333 International Units (IU)**
- **0.3 IU = 0.001 mg RE = 1 g RE**
- **1 µg RE = 3.33 IU**
- **1 µg retinyl palmitate = 0.55 µg retinol**
- **1 µg retinyl acetate = 0.66 µg retinol**
- **1 mg = 1000 µg**

[1] Laillou A, Renaud C, Berger J, Moench-Pfanner R, Fontan L, Avallone S. Assessment of a portable device to quantify vitamin A in fortified foods (flour, sugar, and milk) for quality control. Food Nutr Bull. 2014 Dec;35(4):449-57. doi: 10.1177/156482651403500407. PMID: 25639130.

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