iCheck Fluoro

Measuring Vitamin A in Dry Vitamin Premix and Preblend

1. Method Principle and Application

iCheck Fluoro is a portable fluorometer for quantitative determination of the vitamin A content in vitamin premixes, preblend, sugar, flour and milk. The method is based on the fluorescence of the retinol molecule (excitation at 335 nm, emission at ≥400 nm). The device algorithm calculates vitamin A concentration in µg retinol equivalents (RE) per liter (µg RE/L). The measurement range of iCheck Fluoro is 50 – 3000 µg RE/L.

2. Working with Highly Concentrated Samples

If the expected vitamin A 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 and preblend, 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

- Use an analytical balance!
- Ensure your balance is well-calibrated.
- Record the exact amounts of samples weight and diluted volume for dilution factor calculation.
- Ensure your diluted sample is homogenous.

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3. Vitamin A in Premix and Preblend

Vitamin A (retinol) in dry premix and preblend is typically in the esterified form of retinyl palmitate or retinyl acetate encapsulated in gelatin or starch to improve stability. Before starting the measurement, confirm the vitamin A formulation in your sample – this will affect the sample preparation process.

4. iCheck Fluoro Performance with Premix

iCheck Fluoro performance is assessed following a standardized process that combines assessment of precision, trueness and a comparison to a reference method (i.e., high-performance liquid chromatography). The detailed description of this process is provided in the iCheck Fluoro Performance Guide.

Performance of iCheck Fluoro with premix has been assessed in internal validations. Below is a table detailing the observed precision and recovery.

Table 1. iCheck Fluoro Performance with Premix Samples

Sample Type	Recovery (at 500-2000 µgRE/L dilutions)	Coefficient of Variation
Vitamin Premix for Sugar (250,000 IU/g as retinol palmitate)	106%	±1%
Vitamin Premix for Flour (21,000 IU/g as retinol palmitate)	90%-96%	Max. ±14%

5. Analyzing Dry Premix and Preblend Containing Vitamin A

Based on expected vitamin A concentration, sample weight and dilution factor should be adapted so that the diluted sample concentration is within the linear measurement range of iCheck Fluoro (50 – 3000 µg RE/L). Follow the instructions below to measure retinol in premix and preblend with iCheck Fluoro.

Table 2. Dilution of Premix and Preblend Containing Vitamin A

Sample Type	Expected Retinol Conc. [µg/g]	Expected Vit.A Conc. [IU/g]	Dilution	Sample Weight [g]	Volume of Water [mL]	Expected diluted sample concentration [µg RE/L]
Dry Vitamin Premix or Preblend	50 000	1667	1 : 30 000	**0.03**	1 000	1667
	25 000	833	1 : 25 000	**0.04**	1 000	1000
	10 000	333	1 : 10 000	**0.10**	1 000	1000
	5 000	167	1 : 5 000	**0.20**	1 000	1000

- Weigh in the premix or preblend according to Table 2. Ensure you have a well calibrated analytical balance. The balance supplied with your iCheck Fluoro is not recommended for the weight below 0.1g.
- Dilute premix to 1000 mL with distilled or bottled water and 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 sample is standing still, the 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.

6. 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 original sample vitamin A concentration, you must first calculate the dilution factor according to the following formula:

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

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

$$Vitamin\ A\ in\ sample\ \left(mg\frac{RE}{kg}\right) = iCheck\ Fluoro\ result(\mu g\frac{RE}{L})\ x\ DF/1000$$

7. Vitamin A Unit Conversion

iCheck Fluoro displays vitamin A concentration in retinol equivalents (RE), an arbitrary unit for measurement of vitamin A activity. 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**

For technical support email us: **support@bioanalyt.com**

Last Update: March 2026