

iCheck Chroma 3

Measuring Vitamin A in Premix for Oil

1. Method Principle and Application

The [iCheck Chroma 3](#) is a portable photometer for quantitative determination of the vitamin A content in edible oil in minutes. The method detects vitamin A in based on its reaction with antimony trichloride (SbCl_3). Transient, blue-colored complex is formed and measured at 610 nm, 525 nm and 450 nm. The device algorithm calculates vitamin A concentration in mg retinol equivalents (RE) per kilogram (mg RE/kg). The measurement range of iCheck Chroma 3 is 3.0 – 30.0 mg RE/kg.



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, 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 the sample amount is too low to be accurately weighed in.
- Ensure your diluted sample is homogenous.

3. Vitamin A in Oil Premix

Vitamin A in commercial fortification premix for edible oil is usually supplied as retinyl palmitate at concentrations of 1.7M IU/g and 1.0M IU/g. This document details the dilution protocol of premix containing vitamin A at both concentrations. The method is suitable for quantification of vitamin A as retinol palmitate and retinol acetate.

4. iCheck Chroma 3 Performance

iCheck Chroma 3 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 Chroma 3 Performance Guide](#).

Below is a summary of internal validation data on recovery and variability when using premix for oil containing 1.0 M IU/g vitamin A, as well as retinol palmitate analytical standard with 99.5% purity. The dilution of the samples was done with high purity sunflower or rapeseed oil.

Table 1. iCheck Chroma 3 performance with vitamin premix for edible oil and retinol palmitate analytical standard

Sample	Recovery	Coefficient of Variation
1,000,000 IU/g Vitamin A premix for edible oil	93%-97%	±6%
1,700,000 IU/g Vitamin A premix for edible oil	105%-116%	±6%
Retinol palmitate, analytical standard (99,5%)	105%-109%	±6%

5. Analyzing Vitamin A in Premix for Oil with iCheck Chroma 3

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 Chroma 3 (3 – 30 mg RE/kg). Due to high concentration of vitamin A in premix, the dilution should be performed in two steps. Record the exact weight of premix, as well as that of unfortified oil used for accurate dilution factor calculation.

The dilution should be performed using high purity unfortified clear refined oil, such as sunflower or rapeseed oil.



Follow the instructions below to measure vitamin A in premix for oil with iCheck Chroma 3.

Table 2. Serial Dilution of Oil Premix for Vitamin A Quantification with iCheck Chroma 3

	Expected Vitamin A Concentration [IU/g]	Dilution	Premix Sample Weight [g]	Unfortified Oil Weight [g]	Diluted premix concentration [IU/g]	Diluted premix concentration [mg RE/kg]
1.0 M IU/g						
Step 1	1 000 000	1 : 200	0.5	99.5	5 000	1 500
Step 2	5 000	1 : 100	1.0	99.0	50	15.0
1.7 M IU/g						
Step 1	1 700 000	1 : 333	0.3	99.7	5 105	1 531
Step 2	5 105	1 : 100	1.0	99.0	51	15.3

- **STEP 1:** Weigh in the appropriate amount of the premix sample according to Table 2 and Step 1.
- Dilute premix with fresh unfortified refined oil (e.g., rapeseed or sunflower oil) according to Table 2 and Step 1.
- Shake the premix solution vigorously for 10 seconds, let it rest for 5 minutes, then shake again for 10 seconds.
- **STEP 2:** Weigh the appropriate amount of the premix solution from Step 1 according to Table 2 and Step 2.
- Dilute the solution again with fresh unfortified refined oil (e.g., rapeseed or sunflower oil) according to Table 2 and Step 2.
- Shake the premix solution vigorously for 10 seconds, let it rest for 5 minutes, then shake again for 10 seconds.
- The sample is now ready for measurement. Ensure the premix solution is homogenous and proceed as described in the [iCheck Chroma 3 User Manual](#).



7. Dilution Factor (DF) Calculation

The value displayed on iCheck Chroma 3 after measurement will reflect the concentration of vitamin A in the diluted sample. To obtain the original premix vitamin A concentration, you must first calculate the dilution factor according to the following formula:

$$\text{Step 1} = \frac{\text{Total sample solution weight (g)}}{\text{Oil premix weight (g)}}$$

$$\text{Step 2} = \frac{\text{Total sample solution weight (g)}}{\text{Premix solution weight (g)}}$$

$$\text{DF} = \text{Step 1} * \text{Step 2}$$

Once you have calculated the dilution factor, multiply the iCheck Chroma 3 result by the dilution factor.

$$\text{Vitamin A in premix} \left(\text{mg} \frac{\text{RE}}{\text{kg}} \right) = \text{iCheck Chroma 3 result} \left(\text{mg} \frac{\text{RE}}{\text{kg}} \right) \times \text{DF}$$

8. Vitamin A Unit Conversion

iCheck Chroma 3 displays vitamin A concentration in IU/g and mg retinol equivalents (RE)/kg. 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 µg RE**
- **0.3 IU/g = 1 mg RE/kg**
- **1 mg retinyl palmitate = 0.55 mg RE**
- **1 mg retinyl acetate = 0.66 mg RE**

For technical support email us: support@bioanalyt.com

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