

## **VITAMIN B2 IN WHOLE BLOOD BY FLUORIMETRY - FAST –** **Code Z85110**

### **INTRODUCTION**

**Riboflavin** (or **vitamin B2**) was isolated for the first time in 1927 by Paul Gyorgy. He noted that this substance occurred at high concentrations in milk, so it was initially called lactoflavin. It was also called **vitamin G**.

The clarification of its chemical structure and the discovery of a molecule of ribitol, lead to it's name being changed to Riboflavin.

Riboflavin is a heterocyclic compound obtained from a molecule of flavin which is linked to a chain formed by Ribitol. It is a compound of yellow color slightly soluble in water, stable to heat (cooking determines the inactivation of only 10-20% of the total quantity) and fluorescent when subjected to ultraviolet light.

When struck by light, it determines a photolysis reaction which produces the secondment of a radical ribitol and consequent loss of vitamin action.

Riboflavin is found mainly in foods such as phosphorylated forms. To be absorbed, it is dephosphorylated by specific phosphatases that are found in the small intestine. The vitamin is absorbed to facilitate transport.

Riboflavin is transported in the blood linked to certain plasma proteins, primarily albumin and other globulins such as IgA, IgG and IgM. From the blood it reaches the liver and other tissues where it is later turned in flavin-mononucleotide (FMN) and flavin adenine dinucleotide (FAD), the two coenzyme forms.

The two coenzyme forms are essential components of the flavin enzymes, working from prosthetic groups. They are involved in various redox reactions of the metabolism of carbohydrates, proteins and lipids including: pyruvate decarboxylation, oxidation of fatty acids and amino acids, electron transport during oxidative phosphorylation.

Riboflavin, in nature, is abundant. It can be found in vegetables, yeast, especially in milk, liver, heart, kidney and the white of the egg. In vegetables vitamin content is present mainly during active growth and decreases when the plant stops growing. Even in milk the amount of riboflavin can be variable depending on the type of animal feed ingested. In mammalian tissue, the vitamin is present in the coenzyme form.

Riboflavin is involved in numerous metabolic reactions that can also cover other vitamins. This means that a deficiency of riboflavin can lead to a plurideficiency of other vitamins. In the course of riboflavin deficiency are

inter alia: mitochondrial alterations, inhibition of the conversion of vitamin B6 in its coenzyme forms (with consequent pellagra), increased lipid oxidation, iron deficiency anaemia, increased synthesis of glutathione and consequent decrease of amino acids involved in this process.

At the macroscopic level deficiency may result in a pellagra-like syndrome characterized by mucosal lesions and the epithelium of the eye (corneal vascularization bilateral conjunctivitis and opacity), gastrointestinal tract and areas between skin and mucous membranes (feature is the angular stomatitis).

The subject may experience pain or burning sensation in the lips, tongue or mouth and difficulty swallowing and chewing, paleness, formation of cracking and painful fissures, seborrheic dermatitis. The subject can even have watery, burning and itching eyes and decrease of visual acuity.

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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

|        |                                                 |         |
|--------|-------------------------------------------------|---------|
| N° 003 | Vitamin B2 in whole blood by Fluorimetry - FAST | 06/2024 |
|--------|-------------------------------------------------|---------|

## **TECHNICAL FEATURES**

**Principle of Method** : Vitamin B2 are separated treating the serum/plasma with a suitable precipitant and injected directly in the chromatographic system.

**Recovery** : >99%

**Sensitivity (LLOD)**:  
8 ng/ml FAD  
0,6 ng/ml FMN  
0,6 ng/ml Riboflavin

**Minimum concentration analyzable (LLOQ)**:  
25 ng/ml FAD  
2 ng/ml FMN  
2 ng/ml Riboflavin

**Linearity** :  
25 – 2500 ng/ml FAD  
2 – 500 ng/ml FMN  
2 – 50 ng/ml Riboflavin

**Accuracy intra serie (relative error %) FAD:**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 152 ng/ml | 627 ng/ml |
| 2,3%      | 2,0%      |

**Accuracy inter serie (relative error %) FAD:**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 152 ng/ml | 627 ng/ml |
| 4,0%      | 3,7%      |

**Reproducibility intra serie (coefficient of variation %) FAD:**

| <b>C LLOQ</b> | <b>Cm</b> | <b>Cs</b>  |
|---------------|-----------|------------|
| 127 ng/ml     | 177 ng/ml | 1127 ng/ml |
| 3,9%          | 2,8%      | 2,5%       |

**Reproducibility inter serie (coefficient of variation %) FAD:**

| <b>C LLOQ</b> | <b>Cm</b> | <b>Cs</b>  |
|---------------|-----------|------------|
| 127 ng/ml     | 177 ng/ml | 1127 ng/ml |
| 6,0%          | 6,2%      | 3,1%       |

**Coefficient of Correlation R2 + Dev Std**

0,9948 ± 0,0018 FAD

**Accuracy intra serie (relative error %) FMN:**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 5,0 ng/ml | 7,3 ng/ml |
| 2,3%      | 5,6%      |

**Accuracy inter serie (relative error %) FMN:**

| <b>Ci</b> | <b>Cs</b> |
|-----------|-----------|
| 5,0 ng/ml | 7,3 ng/ml |
| 1,9%      | 8,1%      |

**Reproducibility intra serie (coefficient of variation %) FMN:**

| <b>C LLOQ</b> | <b>Cm</b> | <b>Cs</b> |
|---------------|-----------|-----------|
| 4,8 ng/ml     | 5,3 ng/ml | 9,8 ng/ml |
| 14,1%         | 6,6%      | 2,8%      |

**Reproducibility inter serie (coefficient of variation %) FMN:**

| C LLOQ    | Cm        | Cs        |
|-----------|-----------|-----------|
| 4,8 ng/ml | 5,3 ng/ml | 9,8 ng/ml |
| 2,9%      | 2,4%      | 3,9%      |

**Coefficient of Correlation R2 + Dev Std**

0,9694 ± 0,0110 FMN

**Accuracy intra serie (relative error %) Riboflavin:**

| Ci        | Cs        |
|-----------|-----------|
| 4,6 ng/ml | 6,9 ng/ml |
| 2,3%      | 6,0%      |

**Accuracy inter serie (relative error %) Riboflavin:**

| Ci        | Cs        |
|-----------|-----------|
| 4,6 ng/ml | 6,9 ng/ml |
| 2,9%      | 4,7%      |

**Reproducibility intra serie (coefficient of variation %) Riboflavin:**

| C LLOQ    | Cm        | Cs        |
|-----------|-----------|-----------|
| 4,4 ng/ml | 4,9 ng/ml | 9,4 ng/ml |
| 13,3%     | 3,0%      | 1,4%      |

**Reproducibility inter serie (coefficient of variation %) Riboflavin:**

| C LLOQ    | Cm        | Cs        |
|-----------|-----------|-----------|
| 4,4 ng/ml | 4,9 ng/ml | 9,4 ng/ml |
| 2,2%      | 2,7%      | 1,4%      |

**Coefficient of Correlation R2 + Dev Std**

0,9790 ± 0,0057 Riboflavin

**Normal Range in blood :**

|            |                 |
|------------|-----------------|
| FAD        | 137 – 370 ng/ml |
| FMN        | n.d.            |
| Riboflavin | n.d.            |

**Bibliography**

- 1- F.Fidanza et al. (1989), Methods for Thiamine and Riboflavin Nutriture, Internat.J.Vit.Nutr.Res., 59, 40-47  
2-Liquid Chromatographic Determination of B2Vitamins in Human Plasma and Whole Blood  
Jean-Yves Hautem, Claire Morel, Rémy Couderc and Fathi Moussa

**Components of the kit :**

All the reagents are ready to use and stable 3 years at 2–8 °C.

**Reagent A – Precipitant Solution, 1 x 30 ml**

**Reagent B – Diluting Solution, 1 x 30 ml**

**Calibrator in blood, 2 x 1 ml**

**Code Z85116 (packed separately – see data sheets)**

**Reagent M – Mobile Phase, 3 x 500 ml**

**200 pcs amber Eppendorf**

**Minimum Instrumental equipment required:**

Instrument HPLC isocratic with loop of 20 µl  
Fluorimetric Detector λ<sub>EX</sub>=465 nm λ<sub>EM</sub>=525 nm (B2)

**Blood Collection:**

Take 3 ml of blood in a test tube with EDTA as an anticoagulant.

## **ANALYTICAL PROCEDURE**

### **STEP 1** : Samples Preparation

Dispense in amber Eppendorf of 1,5 ml with cap (included in kit) :

|                                         | <b>Blanck</b> | <b>Calibrator</b> | <b>Sample</b> |
|-----------------------------------------|---------------|-------------------|---------------|
| <b>Water HPLC grade</b>                 | 200 µl        |                   |               |
| <b>Calibrator</b>                       |               | 200 µl            |               |
| <b>Sample</b>                           |               |                   | 200 µl        |
| <b>Reagent A – Precipitant Solution</b> | 300 µl        | 300 µl            | 300 µl        |

**Vortex for almost 30 sec.**

**STEP 2** : let stand at 2-8 °C for 10 minutes

**STEP 3** : Centrifuge at 14.000 rpm for 10 minutes

**STEP 4** : Transfer 300 µl of supernatant in an amber eppendorf.

**STEP 5** : Add at each eppendorf 300 µl of **Reagent B – Diluting Solution.**

**Vortex for 10 sec.**

**STEP 6** : Transfer about 200 µl of supernatant in an amber glass vial with insert

**N.B.: at this step, the sample is stable 1 day at 2-8 °C**

### **INJECTION** :

- Inject 20 µl of solution in HPLC system.

## **VITAMIN B2 IN WHOLE BLOOD BY FLUORIMETRY – Warnings**

### **PARAMETERS OF FLUORIMETRIC DETECTOR**

|                |        |
|----------------|--------|
| $\lambda_{EX}$ | 465 nm |
| $\lambda_{EM}$ | 525 nm |
| SENSIBILITY    | HIGH   |

### **HPLC COLUMN PROTECTION**

To save the analytical column Reverse Phase ZORBAX Eclipse Plus C18 (100 x 4,6 mm, 3,5  $\mu$ m), the use of Javelin Colfilters (1 x 10 pcs.) cod. S90199511 is obligatory.

### **HPLC COLUMN CONDITIONING**

Install a new analytical C<sub>18</sub> Reversed-Phase column ZORBAX Eclipse Plus C18 (100 x 4,6 mm, 3,5  $\mu$ m).

Disconnect the detector and filter 30 ml of **H<sub>2</sub>O : Acetonitrile (20 : 80 v / v)** set flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Flux 30 ml of a solution of **H<sub>2</sub>O : Acetonitrile (80 : 20 v / v)** at flow of 1,0 ml/minute. **Don't recycle the washing solutions.** Condition the column with the mobile phase at a flow of 1,0 ml/minute and discharge the first 20 ml. Finally inject the Chemical Standard and verify the quality of the HPLC run.

**It's NOT possible to make analysis at recycling phase.** If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

### **COLUMN CLEANING**

Disconnect the detector. Flux 15 ml of **H<sub>2</sub>O : Acetonitrile or Methanol (80 : 20 v / v)** set flow of 1,0 ml/minute and discharge. Flux a solution of **H<sub>2</sub>O : Acetonitrile or Methanol (30 : 70 v / v)** for 30 minutes discharge it. **It is recommended to perform the washing of Analytical Column at the end of each analytical run.** Store the column in a solution of **H<sub>2</sub>O : Acetonitrile or Methanol (30 : 70 v / v)**.

### **INJECTION NEEDLE WASHING**

Wash with a solution of Water / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

### **HPLC PARAMETERS**

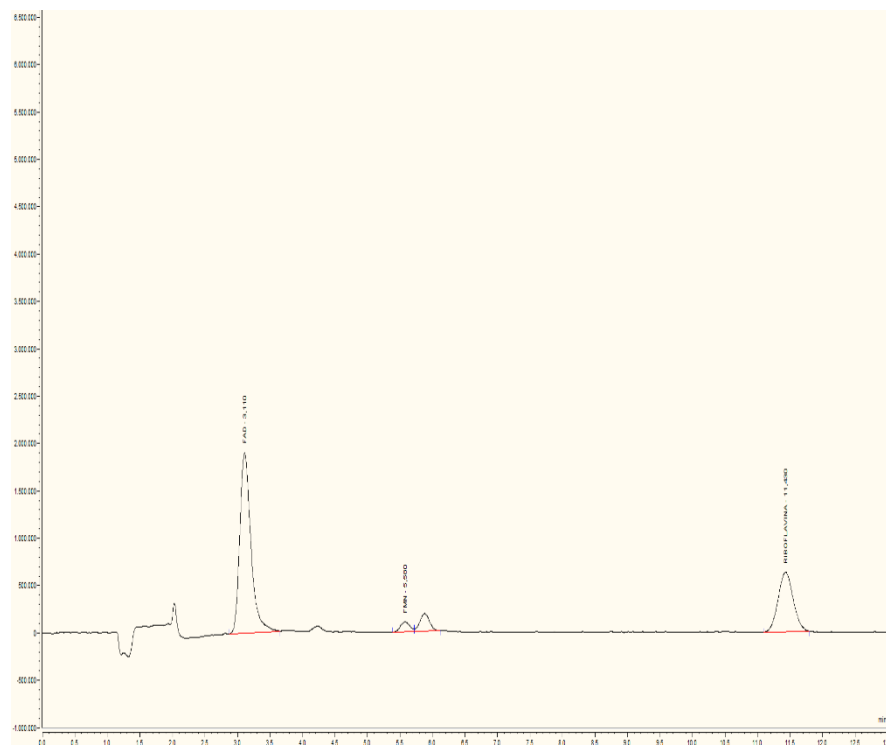
|          |              |
|----------|--------------|
| LOOP     | 20 $\mu$ l   |
| FLOW     | 0,8 ml/min.  |
| PRESSURE | About 60 bar |

### **ACCESSORIES AND CONSUMABLES**

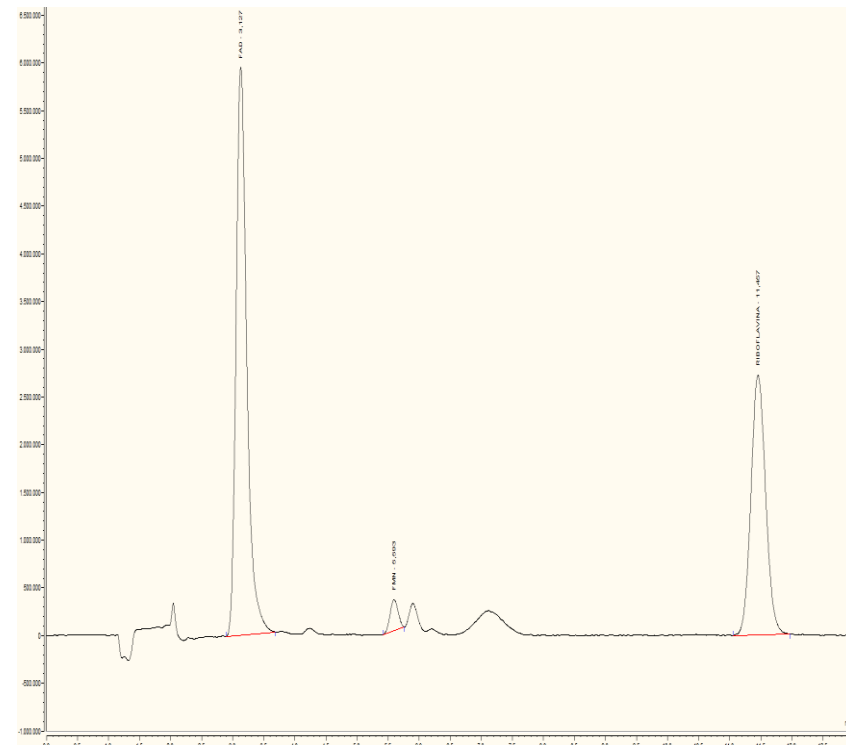
| CODE              | DESCRIPTION                                                           | PACKAGING    |
|-------------------|-----------------------------------------------------------------------|--------------|
| <b>Z85116</b>     | Calibrator in whole blood for Vitamin B2                              | 4 x 1 ml     |
| <b>Z85019</b>     | Control in whole blood for Vitamin B1/B2/B6 – Levels 1 and 2          | 2 x 5 x 1 ml |
| <b>Z85111</b>     | Mobile Phase                                                          | 4 x 500 ml   |
| <b>Z959961902</b> | Zorbax Eclipse Plus C 18 (100 x 4,6mm –3,5 $\mu$ m) Analytical Column | 1 Pc         |
| <b>S29004U</b>    | Amber glass vials of 2 ml                                             | 1 x 100 Pcs  |
| <b>S24717</b>     | Glass Insert 100 $\mu$ l for amber vials of 2 ml                      | 1 x 100 Pcs  |
| <b>S51820717</b>  | Caps for amber glass vials of 2 ml                                    | 1 x 100 Pcs  |

# VITAMIN B2 IN WHOLE BLOOD

(Cromatogrammi di riferimento)



|                 |                                  |           |  |
|-----------------|----------------------------------|-----------|--|
| <b>Fig. 1 :</b> | <b>Calibrator in whole blood</b> |           |  |
|                 | R.T. 3.1 FAD                     | 150 ng/ml |  |
|                 | R.T. 5.5 FMN                     | 5 ng/ml   |  |
|                 | R.T. 11.4 Riboflavin (B2)        | 17 ng/ml  |  |



|                 |                                         |           |  |
|-----------------|-----------------------------------------|-----------|--|
| <b>Fig. 2 :</b> | <b>Control in whole blood – Level 2</b> |           |  |
|                 | R.T. 3.1 FAD                            | 350 ng/ml |  |
|                 | R.T. 5.5 FMN                            | 8 ng/ml   |  |
|                 | R.T. 11.4 Riboflavin (B2)               | 28 ng/ml  |  |