DRG® Vitamin B2 (Riboflavin) (BIO-4887)

USA: RUO

Revised 6 Aug. 2010 rm (Vers. 2.1)

INTENDED USE

Vitamin B_2 is a microtiter plate test kit based on a microbiological assay which measures the total Vitamin B_2 content in serum. The test kit contains all required reagents, e.g. standard, medium and microtiter plate coated with a specific microorganism, sufficient for 96 determinations including standard curves. An ELISA reader is required for evaluation of the Vitamin B_2 content.

In the United States, this kit is intended for Research Use Only.

INTRODUCTION

Vitamin B_2 (riboflavin), as flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), acts as an essential coenzyme in many oxidation-reduction reactions. Flavins are critical for the metabolism of carbohydrates, fats, and proteins. FAD is part of the electron transport (respiratory) chain, which is central to energy production. In conjunction with cytochrome P-450, flavins also participate in the metabolism of drugs and toxins.

Vitamin B_2 -Deficiency

Riboflavin is unique among the water soluble vitamins in that milk and dairy products make the greatest contribution to its intake in Western diets. Biochemical signs of depletion arise within only a few days of dietary deprivation. Poor riboflavin status in Western countries seems to be of most concern for the elderly and adolescents, despite the diversity of riboflavin-rich foods available. Deficiency results in oral, ocular, cutaneous, and genital lesions.
Primary riboflavin deficiency is associated with inadequate consumption of milk and other animal products. Secondary deficiencies are most common in chronic diarrheas, liver disease, chronic alcoholism, and postoperative situations in which nutrient infusions lack supplementary vitamins.

Indications for Vitamin-B_2-determination:

- Chronic diarrhoea
- Preeclampsia
- Alcohol abuse
- Anorexia
- Lactose intolerance
- Hypothyroidism
- Diabetes mellitus

PRINCIPLE OF THE TEST

Serum samples are transferred in the wells of a microtiter plate [Plate] coated with *Lactobacillus rhamnosus*. The addition of Vitamin B_2 in either standards or samples gives a Vitamin B_2-dependent growth response until Vitamin B_2 is consumed. After incubation at 37°C for 48 h, the growth of *Lactobacillus rhamnosus* is measured turbidimetrically at 610 - 630 nm (alternatively at 540 - 550 nm) in an ELISA-reader and a standard curve is generated from the dilution series.

The amount of Vitamin B_2 is directly proportional to the turbidity.

DRG International Inc., USA

Fax: (908) 233-0758 • E-mail: corp@drg-international.com • **Web:** www.drg-international.com

DRG

DRG® Vitamin B2 (Riboflavin) (BIO-4887)

USA: RUO

Revised 6 Aug. 2010 rm (Vers. 2.1)

MATERIAL SUPPLIED

	Kit Components	Quantity
Plate	One Lactobacillus rhamnosus-precoated microtiter plate, ready to use	12 x 8 wells
Dilution Water	Water 30 ml	4 x
Assay Medium	Vitamin B_2 assay medium	4 x
Standard	Vitamin B_2 standard	4 x
Control	Vitamin B_2 control	4 x
Foil	Cover plastic foil	4 x
Holder	Replacement holder for 96-well plates	1 x

MATERIAL REQUIRED BUT NOT SUPPLIED

- Incubator with a dark incubation chamber, 37 °C
- Water bath (90°C - 100°C)
- ELISA-Reader 610 - 630 nm (540 - 550 nm)
- Micropipette 20 - 200 µl
- Micropipette 100 -1000 µl
- Micropipette tips to deliver 20 - 200 µl and 100 -1000 µl, sterile
- Pipettes of 5 and 10 ml
- 1.5 - 2 ml reaction vials, sterile
- 0.2 µm sterile polyethersulfone filter with a sterile tip
- 15 ml centrifugal tubes, sterile (e.g. Falcon tubes)
- Biocentrifuge (10 000 x g)

PREPARATION AND STORAGE OF REAGENTS

- Store test kit / reagents at 2-8°C.
- Prepare reagents freshly and use immediately after preparation. Discard remaining unused reagents and waste in accordance with country, federal, state, and local regulations.
- Put unused reagents (standard, medium) in the test kit and store at 2-8°C.
- Store unused strips in the original package with dry bag securely closed at 2-8° C to prevent contamination or moisture exposure.
- No warranty can be given after the expiry date (see label of test package).
- To run assay more than once, ensure that reagents are stored at conditions stated on the label. Prepare only the appropriate amount necessary for each assay. The kit can be used up to 4 times within the expiry date stated on the label.

DRG International Inc., USA

Fax: (908) 233-0758 • E-mail: corp@drg-international.com • **Web:** www.drg-international.com

PRECAUTIONS

- As the test is based on a microbiological method, the general guidelines for sterile work must be observed as far as possible, (work in a sterile bench, PCR-Hood, use of sterile instruments or equipment).
- GLP (Good Laboratory Practice)-guidelines should be observed.
- Water quality is extremely important. Only the water delivered with the test kit [Dilution Water] should be used for medium dilution [Assay Medium], standard reconstitution as well as for sample preparation.
- It is essential to run a standard curve for each separate assay.
- It is recommended to run a duplicate standard curve as well as a sample analysis.
- If a higher dilution results in a higher measured value, inhibitors like antibiotics might be present.
- Reagents should not be used beyond the expiration date shown on kit label.
- Wear gloves during the test.
- Used microtiter plates and materials that have been in contact with donor‘s samples should be handled and disposed as potentially infectious.
- Signs for reagent damage: The highest standard should have an absorption higher than 0.6 Extinktion units (A_{630}nm > 0,6)

SAMPLE PREPARATION

Notes

- Donor serum is used for analysis.
- Original samples should be kept light-protected at 2-8°C until measurement. The samples are stable for 8 hours at 2-8°C in the dark. For longer storage, samples should be frozen and kept at -20°C.
- Hemolytic samples may give erroneous results and should not be used for analysis. Lipemic samples should be centrifuged at 13 000 x g before assaying to obtain fat free serum as far as possible.
- Samples should be centrifuged (5 min at 10000 g) prior to measurement and the resulting supernatant used in the test.

1.1 Sample pretreatment

Centrifuged serum samples can be added undiluted.

ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test.
- The assay should always be performed according the enclosed manual.

1.2 Test preparations

Take as many microtiter strips as needed from kit.

Return unused strips and any unused test kit component to the original foil bag, reseal them together with the desiccant provided, and put in the refrigerator.

Bring all necessary reagents to room temperature.

Water [Dilution Water] for medium [Assay Medium], control [Control] and Standard

Push the lid up, pull it back to the rim of the glass and then remove the entire seal by turning.

Assay medium [Assay Medium]

The medium must be freshly prepared before each test.

- Take the dry bag out of medium vial [Assay Medium] by tweezers, shake off and discard.
- Add 10 ml of water [Dilution Water] to the assay medium [Assay Medium], securely close the bottle and shake well. The amount is sufficient for 6 strips.
- Heat the bottle with medium [Assay Medium] in a water-bath at 90 - 100 °C for 5 min, while shaking well at least twice. It is important to make sure that the medium bottle [Assay Medium] is firmly closed at all times.
- Quickly cool the medium bottle [Assay Medium] to under 30 °C.
- Filter 10 ml medium [Assay Medium] sterilely with a 0.2 µm filter in a centrifuge test tube (e.g. 15 ml, Falcon).

Standard

Before the test, freshly prepare the standard curve solutions:

- Open the bottle of standard, place the screw-top lid upside-down on the work bench.
- Add x ml (x = see QS test kit data sheet) water [Dilution Water] from the test kit to the standard bottle, close the bottle and shake (= standard concentrate).
- Add water [Dilution Water] into 6 sterile reaction vials (capacity 1.5 – 2.0 ml) and then pipet the standard concentrate to the vials. Prepare a standard curve using the following scheme:

Vitamin B2 [µg/l]	Water [Dilution Water] [µl]	+	Standard [Standard] [µl]	=	Total volume [µl]
Blank: 0	900	+	0	=	900
Standard 1: 5	950	+	50	=	1000
Standard 2: 10	900	+	100	=	1000
Standard 3: 20	400	+	100	=	500
Standard 4: 30	350	+	150	=	500
Standard 5: 60	200	+	300	=	500

Control

The control must be freshly prepared before the test.

- Open the bottle of control [Control], remove seal. Dispose of screw-top lid and seal.
- Add 5 ml water [Dilution Water] from the test kit to the control bottle [Control], close the bottle and dissolve by vortexing the bottle (= control).
- Treat the control afterwards as the sample is treated.
- Pipette 150 µl of the reconstituted control [Control] into each well. We recommend to run a duplicate.
- For the concentration of the control [Control] please see control specification.

1.3 Test Initiation

- Take as many microtiter strips as needed from the kit and put them in the second microtiter strip holder. Return unused strips to the original foil bag, reseal them together with the desiccant provided, and store at 2-8° C to prevent contamination or moisture exposure.
- A medium solution is sufficient for 6 strips.
- Put 150 µl Vitamin B_2 assay medium [Assay Medium] in the cavities.
- Add 150 µl standard, control [Control], respectively, sample in the cavities. Pre-rinse the pipette tip with standard, control and sample solution respectively.
- Carefully seal the plate with plastic foil. Important: the cavities must be made airtight by pressing down with the hand!
- Keep at **37 °C for 48 hrs** in an incubator.

1.4 Measurement

- Securely press the foil down with the hand.
- Upturn the plate [Plate] onto a tabletop and shake the germination well.
- Turn the plate [Plate] over again and carefully remove the foil, beginning with the lower, left corner and pulling diagonally backwards at an angle of 180°.
- Remove air bubbles in the cavities using a pipette tip or a needle.
- Read turbidity in an ELISA-Reader at E 610 - 630 nm (alternatively at 540 - 550 nm).

Please note

- After 48 h incubation time, the microtiter plate may be stored for a maximum of 48 hrs in the refrigerator before measuring the turbidity.
- To prevent time-loss through public holidays or weekends, the microtiter plate may also be evaluated after 60 hrs incubation.

REFERENCES

Powers HJ (2003) Riboflavin (vitamin B-2) and health.
Am J Clin Nutr 77(6):1352-60. Review

GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for in vitro use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the results of the test. DRG cannot, therefore, be held reliable for any damage resulting from this.