

Total content (natural + added vitamin B2) in food (see 6.5. manual)

## Sample extraction

- weigh exactly 1 g (ml) of homogenized sample into a 50 ml centrifuge vial, add 20 ml redist. or deionized water, shake and adjust to pH 4.5 with HCl
- alternatively instead of water a citrate buffer can be used for the extraction (no pH adjustment is necessary): to 1 g sample add 20 ml citrate buffer pH 4.5 and shake
- add 300 mg taka diastase, 10 mg acid phosphatase of potato, shake well and incubate 1 h at 37 °C (98.6 °F) in the dark (shake at times); fill up to 40 ml with redist. or deionized water and heat 30 min at 95 °C (203 °F) in a water bath; chill down quickly to below 30 °C (86 °F)
- transfer 1 ml of the sample extraction in a 1.5 ml sterile reaction vial and centrifuge 5 min (greater than 8,000 x g)



## Assay - medium

- remove the desiccant using tweezers
- add 10 ml of sterile water (from test kit) to the medium bottle
- heat 5 min at 95 °C (203 °F), chill down quickly to below 30 °C (86 °F)
- filter through a 0.2 µm filter into a sterile 15 ml centrifuge vial



## Sample dilution

- calculate sample dilutions
- fill out microtiter plate manager
- dilute the clear supernatant from the extracted sample with sterile water (from test kit) in 1.5 ml reaction vials



## Standard curve

- reconstitute standard with x ml sterile water (from test kit), x = see bottle
- shake and prepare standard curve (take 1.5 ml sterile reaction vials)

standard curve in mg / 100 g (ml)	sterile water in µl		standard concentrate in µl		total volume in µl
blank: 0	900	+	0	=	900
standard 1: 0.04	900	+	100	=	1000
standard 2: 0.08	400	+	100	=	500
standard 3: 0.12	350	+	150	=	500
standard 4: 0.16	300	+	200	=	500
standard 5: 0.24	200	+	300	=	500

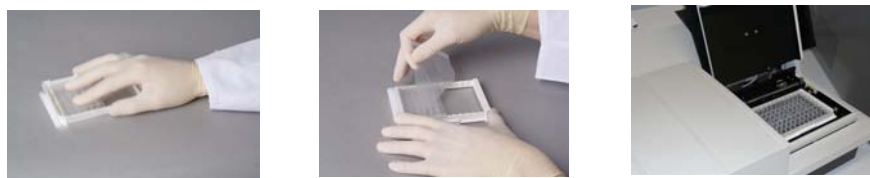


**Microtiter plate**

- transfer the required strips into the additional holder
- return the unused strips together with the desiccant to the foil bag and seal it well
- pipette assay - medium: **150 µl**
- pipette standard / diluted sample: **150 µl**  
(for each standard / diluted sample solution use new tip)
- cover the strips well with adhesive foil
- incubate **44 - 48 h at 37 °C (98.6 °F)** (in the dark)

**Evaluation**

- prepare data file software, put in data (standards, samples, dilutions)
- press down the adhesive foil once more
- place the microtiter plate upside down on a table and dissolve the microorganisms thoroughly by shaking the plate on the surface of the desk
- invert the plate to the regular position and remove the adhesive foil (**hold strips in the frame**)
- disturb the bubbles by means of tip or needle
- read out (610 - 630 nm, alternatively at 540 - 550 nm), use 4 - parameter software (blank has not to be substrated)

**Disposals needed**

- sterile graduated centrifuge vials, 15 and 50 ml
- sterile reaction vials, 1.5 ml
- sterile tips for micropipettes, 20 - 200 µl and 100 - 1000 µl
- sterile filters polyethersulfon 0.2 µm with syringe

**Reagents additionally needed**

- redist. or deionized water
- taka diastase, aspergillus oryzae; (e.g. Fluka 86250)
- phosphatase, acid, Type II: from potato (e.g. Sigma P3752)
- citrate buffer pH 4.5 (weigh 1.5 g citric acid monohydrate (e.g. Roth 5110.3) in a 100 ml beaker with magnetic stirrer; solve the citric acid with about 50 ml redist. or. deionized water under stirring; thereafter add 12 ml NaOH 1 mol / l (or 0.48 g NaOH); the pH should be 4.5 (correct with HCl 0.1 mol / l); transfer the solution quantitatively with redist. or deionized water in a 100 ml volumetric flask and fill up with redist. or deionized water to the mark; the buffer can be stored 3 days at 2 - 8 °C (35.6 - 46.4 °F)
- HCl 0.1 mol / l and 1.0 mol / l

- read the test insert of VitaFast® Vitamin B2 (Riboflavine) carefully
- the extraction procedure above is described for the total content of vitamin B2 in food
- further extraction procedures of added vitamin B2 in liquid samples (6.1), of added vitamin B2 in fruit gums and candies (6.2), of added vitamin B2 in capsules, pills and vitamin mixes (6.3), of added vitamin B2 in cereals, baby food, bread, flour (6.4) and of total vitamin B2 content in yeasts (6.6) are described in the manual