

Cat. No. 985 822



**Test 8-22 05.05**  
**NANOCOLOR® BOD<sub>5</sub>**

**Method:**

Tube test for the determination of the biochemical oxygen demand in 5 days (BOD<sub>5</sub>) by using the diluting principle according to the German Standard Method **DIN 38409-H51**. The incubation of the samples is carried out in Winkler oxygen flasks. The determination of oxygen dissolved in water at day 0 and after 5 days is carried out similarly to the Winkler Method **DIN EN 25813-G21** by photometric evaluation of iodine-colour.

Range:	2-3000 mg/l O <sub>2</sub>	
Factor:	007.0	007.6
Wavelength (HW = 5-12 nm):	436 nm	445 nm
Reaction time:	5 days	
Reaction temperature:	(20 ± 1) °C	

**Contents of reagent set:**

3 empty test tubes  
15 ml BOD<sub>5</sub> R1 15 ml BOD<sub>5</sub> R2 30 ml BOD<sub>5</sub> R3

**Hazard warning:**

BOD<sub>5</sub> R2 contains sodium hydroxide solution 25%. BOD<sub>5</sub> R3 contains sulphuric acid 60%.  
R35 Causes severe burns. S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.  
S37/39 Wear suitable protective gloves and eye protection. Safety data sheets are available on the Internet at www.mn-net.com or can be obtained from MACHEREY-NAGEL upon request.

**Storage:**

The test kit must be stored under dry conditions at room temperature (+15 °C to +25 °C). Please observe expiry date.

**Interferences:**

Changes in pH-value, accumulation of special microbial metabolites and compounds, which are toxic to microorganisms (e. g. mycotoxines, free chlorine, heavy metals) can cause a decrease of substrate metabolism and a reduction of the oxygen consumption. Iron(II) salts, sulphur dioxide and sulphur hydrogen consume oxygen and falsify the BOD<sub>5</sub>-results, meaning they cause false negative results, also. If algae or nitrified microorganisms are present, increased results could occur.

**Sample preparation:**

At the beginning, the sample is adjusted to room temperature. Then the pH-value is checked. The pH-value of the sample should be between pH 6 and 8, and has to be adjusted, if necessary. If, in this case, a precipitate has been developed, the sample should be homogenized very well or filtrated (membrane filtration kit including CHROMAFIL® membrane filters, pore size 1.2 µm, Cat. No. 916 511). In case of samples containing algae, filtration may also be necessary in order to avoid exaggerated results. Remove free and/or bounded chlorine by addition of sodium sulphite.

*Remark:* Store the sample in a tightly closed bottle full to the brim at a temperature of 0 °C to +4 °C immediately after taking the sample until carrying out the analysis. Start the BOD<sub>5</sub> determination as soon as possible or within 24 hours of taking the sample. Samples may also be frozen to keep longer. Homogenise frozen samples after thawing and always use inoculated BOD<sub>5</sub>-Nutrient Solution (see BOD<sub>5</sub>-Nutrient Mixture, Cat. No. 918 994 or BOD<sub>5</sub>-Nutrient Mixture PLUS, Cat. No. 918 995).

**Diluting water, BOD<sub>5</sub>-Nutrient Solutions and inoculating water:**

Details on the preparation and handling of diluting water are given in the BOD<sub>5</sub>-Accessories Set (Cat. No. 916 918). For the use and application of BOD<sub>5</sub>-Nutrient Solutions and inoculating water, refer to the instructions for reagent sets BOD<sub>5</sub>-Nutrient Mixture (Cat. No. 918 994) or BOD<sub>5</sub>-Nutrient Mixture PLUS (Cat. No. 918 995). Make sure to observe the data specified there.

**Determination of BOD<sub>5</sub>:**

Requisite accessories: BOD<sub>5</sub>-Accessories Set (Cat. No. 916 918), BOD<sub>5</sub>-Nutrient Mixture (Cat. No. 918 992) or BOD<sub>5</sub>-Nutrient Mixture PLUS (Cat. No. 918 995), graduated cylinders (volume 100 ml and 500 ml), piston pipettes with tips, equipment for incubation with thermostat for (20 +/- 1) °C (e. g. water bath or incubator) or as an alternative a dark room with a room temperature of about 20 °C.

**Step 1: Control (oxygen consumption of the diluting water)**

Fill in a 1 l laboratory flask (BOD<sub>5</sub>-Accessories Set, Cat. No. 916 918)  
**500 ml aerated diluting water** and  
**2,5 ml nutrient solution** (1.25 ml R1 + 1.25 ml R2 from reagent set BOD<sub>5</sub>-Nutrient Mixture, Cat. No. 918 994 or BOD<sub>5</sub>-Nutrient Mixture PLUS, Cat. No. 918 995), close the vessel and mix to enrich the oxygen content by shaking vigorously for a few seconds (**Control**).

Open  
1 Winkler oxygen flask and  
1 test tube, wash both with several millilitres of the control and fill to the brim without letting air bubbles in.  
Close the Winkler oxygen flask, **without** letting **air bubbles** in, by slowly pressing in the obliquely cut glass stopper and incubate in a water bath or in an incubator for **5 days** at **(20 +/- 1) °C** in the dark.  
Close the test tube **without** letting **air bubbles** in and immediately start the measurement of dissolved oxygen according to **step 3**.

**Step 2: Sample dilutions**

Depending on the expected BOD<sub>5</sub> of a sample, the most suitable dilution in accordance to the following table must be prepared in a 1 l-laboratory flask (BOD<sub>5</sub>-Accessories Set, Cat. No. 916 918). If there is no experience regarding the expected BOD<sub>5</sub> of a sample, at least two, preferably three, different dilutions of this sample should be prepared to assure accuracy of the determination. For more reliable results, we recommend **duplicate determinations**.

Expected BOD <sub>5</sub> [mg/l O <sub>2</sub> ]	Dilution	Examples for typical waters	Sample [ml]	Aerated diluting water [ml]	Nutrient Solution* [ml]	
					R1	R2
< 5	1: 1	R	500	0	1.25	1.25
4 - 12	1: 2	R, B	250	250	1.25	1.25
10 - 30	1: 5	R, B	100	400	1.25	1.25
20 - 60	1: 10	B	50	450	1.25	1.25
40 - 120	1: 20	C	25	475	1.25	1.25
100 - 300	1: 50	C, M	10	490	1.25	1.25
200 - 600	1: 100	C, M	5	495	1.25	1.25
400 - 1200	1: 200	M, I	2	398	1.0	1.0
800 - 2400	1: 400	I	1	399	1.0	1.0
1000 - 3000	1: 500	I	1	499	1.25	1.25

\*: BOD<sub>5</sub>-Nutrient Mixture (Cat. No. 918 994) or BOD<sub>5</sub>-Nutrient Mixture PLUS (Cat. No. 918 995)

R: River water  
B: Biologically suitable biomass from a sewage plant  
C: Clarified biomass from a sewage plant or mildly polluted industrial waste water  
M: Raw municipal sewage  
I: Heavily polluted industrial waste water

After preparation of the sample dilution based on the above table, close the laboratory flask and mix to enrich the oxygen content by shaking vigorously for a few seconds.

Open

1 Winkler oxygen flask and  
1 test tube, wash both with some millilitres of the sample dilution and fill to the brim without letting air bubbles in.

Close the Winkler oxygen flask, **without** letting **air bubbles** in, by slowly pressing in the obliquely cut glass stopper and incubate in a water bath or in an incubator for **5 days** at **(20 +/- 1) °C** in the dark.

Close the test tube **without** letting **air bubbles** in and immediately start the measurement of dissolved oxygen according to **step 3**.

**Proceed in the same way for all other samples or sample dilutions.**

*Remark:* The added laboratory flask in the BOD<sub>5</sub>-Accessories Set can be used for all preparations of any water samples to be tested (control, sample dilutions). Before using, the flask must be washed thoroughly by using tap water, after every preparation and before every new preparation, respectively.

**Step 3: Measurement of dissolved oxygen**

*Preliminary remarks:*

The added test tubes in the reagent set NANOCOLOR® BOD<sub>5</sub> can be used for all measurements of dissolved oxygen. Before using for a new determination of dissolved oxygen the test tube is directly be emptied down the drain and thoroughly washed with tap water. Additional empty test tubes can be ordered at MACHEREY-NAGEL (Cat. No. 916 80).

**Measurement of dissolved oxygen on day 0:** The measurement of dissolved oxygen in test tubes filled at the beginning of the test (day 0) has to be started immediately.

**Measurement of dissolved oxygen on day 5:** The measurement of the concentration of dissolved oxygen in the incubated Winkler flasks after 5 days of incubation starts with the filling of one empty test tube (for double determinations two empty test tubes) to the brim, with the water sample to be tested (control and sample dilutions). After the filling, the test tubes are carefully closed without letting air bubbles in, and the determination of dissolved oxygen is carried out as in the following chapter "Procedure" described.

**Procedure**

Open test tube, filled with control or sample dilution, and add  
**2 drops BOD<sub>5</sub> R1,**  
**2 drops BOD<sub>5</sub> R2,** close **without** letting **air bubbles** in and shake.  
Wait **2 min.**

Open test tube, add  
**5 drops BOD<sub>5</sub> R3,** close **without** letting **air bubbles** in and shake to dissolve the flakes.  
Clean outside of test tube and perform measurement.

**Measurement with NANOCOLOR® photometers:**

For NANOCOLOR® photometers see manual, test 8-22.  
The measured values are indicated in [mg/l O<sub>2</sub>].

**Photometers of other manufacturers:**

For other photometers check whether measurement of round glass tubes is possible. Verify factor for each type of instrument by measuring standard solutions.

**Each indicated concentration of dissolved oxygen in the display of the photometer after every single measurement at day 0 and after 5 days of incubation must be noticed very carefully. To simplify the evaluation, we recommend the use of the table for evaluation at the back of this application guideline. Please feel free to copy this table for your own personal use.**

**Step 4: Evaluation**

*Important:*

The BOD<sub>5</sub> value is only calculated for samples in which the residual oxygen concentration after 5 days incubation still amounts to at least 2 mg/l O<sub>2</sub> and where the oxygen consumption lies between 2 and 6 mg/l O<sub>2</sub>.

**Oxygen consumption of the diluting water O<sub>c</sub> (control):**

O<sub>c</sub> = O<sub>c0</sub> - O<sub>c5</sub>      O<sub>c0</sub> = oxygen concentration in the control at the beginning of the test (day 0)  
                                    O<sub>c5</sub> = oxygen concentration in the control at the end of the test (day 5)

**Oxygen consumption of the sample O<sub>s</sub> (sample dilution):**

O<sub>s</sub> = O<sub>s0</sub> - O<sub>s5</sub>      O<sub>s0</sub> = oxygen concentration in the sample dilution at the beginning of the test (day 0)  
                                    O<sub>s5</sub> = oxygen concentration in the sample dilution at the end of the test (day 5)

**Calculation of BOD<sub>5</sub>:**

**BOD<sub>5</sub> = V x (O<sub>s</sub> - O<sub>c</sub>) + O<sub>c</sub>**

V = reciprocal value of the sample dilution  
(e. g. sample dilution 1 : 200 ---> V = 200)

**Presentation of the results:**

The BOD<sub>5</sub> is given in mg/l O<sub>2</sub> and noted as follows:

< 10 mg/l O<sub>2</sub>      rounded up to mg/l (e.g. 6.7 mg/l O<sub>2</sub> is rounded up to 7 mg/l O<sub>2</sub>)  
10 - 1000 mg/l O<sub>2</sub>      reported with two significant digits (e.g. 314 mg/l O<sub>2</sub> is reported as 310 mg/l O<sub>2</sub>)  
> 1000 mg/l O<sub>2</sub>      reported with three significant digits (e.g. 1578 mg/l O<sub>2</sub> is reported as 1580 mg/l O<sub>2</sub>)

**Analytical Quality Control:**

NANOCOLOR BOD<sub>5</sub> Standard (Cat. No. 925 82)

The test of the BOD<sub>5</sub> standard must be carried out in accordance with DIN EN 1899-1-H51 using an inoculated BOD<sub>5</sub>-Nutrient Solution (details see BOD<sub>5</sub>-Nutrient Mixture, Cat. No. 918 994 or BOD<sub>5</sub>-Nutrient Mixture PLUS, Cat. No. 918 995).

## Table for evaluation for **NANOCOLOR**<sup>®</sup> BOD<sub>5</sub> - Test 8-22

Sample: \_\_\_\_\_ Date: \_\_\_\_\_

COD [mg/l O<sub>2</sub>]: \_\_\_\_\_ pH-value: \_\_\_\_\_ total Phosphate [mg/l P]: \_\_\_\_\_

Ammonia-N [mg/l NH<sub>4</sub>-N]: \_\_\_\_\_ Nitrite-N [mg/l NO<sub>2</sub>-N]: \_\_\_\_\_ Nitrate-N [mg/l NO<sub>3</sub>-N]: \_\_\_\_\_

### Results of the determination of BOD<sub>5</sub>:

Date of test beginning (day 0): \_\_\_\_\_ Date of test end (day 5): \_\_\_\_\_

<u>Control:</u>	O <sub>C0</sub> [mg/l O <sub>2</sub> ]	O <sub>C5</sub> [mg/l O <sub>2</sub> ]	O <sub>C</sub> [mg/l O <sub>2</sub> ] = (O <sub>C0</sub> - O <sub>C5</sub> )

### Sample dilutions:

Dilution V	O <sub>S0</sub> [mg/l O <sub>2</sub> ]	O <sub>S5</sub> [mg/l O <sub>2</sub> ]	O <sub>S</sub> [mg/l O <sub>2</sub> ] = (O <sub>S0</sub> - O <sub>S5</sub> )	BOD <sub>5</sub> [mg/l O <sub>2</sub> ] = [V x (O <sub>S</sub> - O <sub>C</sub> ) + O <sub>C</sub> ]

∅ BOD<sub>5</sub> [mg/l O<sub>2</sub>]: \_\_\_\_\_

O<sub>C</sub> = oxygen consumption of the control after an incubation period of 5 days  
O<sub>C0</sub> = oxygen concentration in the control at the beginning of the test (day 0)  
O<sub>C5</sub> = oxygen concentration in the control at the end of the test (day 5)  
O<sub>S</sub> = oxygen consumption of the sample dilution after an incubation period of 5 days  
O<sub>S0</sub> = oxygen concentration in the sample dilution at the beginning of the test (day 0)  
O<sub>S5</sub> = oxygen concentration in the sample dilution at the end of the test (day 5)  
V = reciprocal value of the sample dilution (e. g. sample dilution 1 : 200 --> V = 200)