REF 985825

Test 8-25 04.23

NANOCOLOR® BOD_5-TT

Method:
Tube test for determination of the biochemical oxygen demand in 5 days (BOD_5) in the presence of added nutrients according to DIN EN 1899-1-H51. Additionally, a probable influence of nitrification processes is inhibited by *N*-allylthiourea. The incubation of the samples is carried out directly in test tubes. The determination of oxygen dissolved in water is carried out after 5 days in accordance to the Winkler Method DIN EN 25813-G21 by photometric evaluation of iodine-color.

Range:	**2 – 3000 mg/L O_2**	**2 – 3000 mg/L O_2**
Wavelength (HW = 5 – 12 nm):	**436 nm**	**445 nm**
Reaction time:	**5 days**	
Reaction temperature:	**20 ± 1 °C**	

Contents of reagent set:
Box A: 22 test tubes BOD_5-TT

Box B: 1 bottle with 3 mL BOD_5-TT R1
1 bottle with 3 mL BOD_5-TT R2
1 bottle with 6 mL BOD_5-TT R3
23 screw caps

Hazard warning:
Reagent R1 contains manganese(II) chloride 25 – 83 %, reagent R2 contains sodium hydroxide solution 20 – 55 %, reagent R3 contains sulfuric acid 51 – 80 %.
H314 Causes severe skin burns and eye damage.
P260, P280, P301+330+331, P303+361+353, P304+340, P305+351+338, P501 Do not breathe vapors. Wear protective gloves / eye protection. IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water / shower. IF INHALED: Remove person to fresh air and keep comfortable for breathing. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Dispose of contents / container to regulate waste treatment. For further information ask for a safety data sheet.

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, sulfur dioxide and sulfur hydrogen consume oxygen and falsify the BOD_5-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, REF 916511). 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 sulfite.
Remark: *Store the sample in a tightly closed bottle full to the brim at a temperature of 0 – 4 °C immediately after taking the sample until carrying out the analysis. Start the BOD_5 determination as soon as possible or within 24 hours of taking the sample. Samples may also be frozen to keep longer. Homogenize* ***frozen samples*** *after thawing and always use* ***inoculating water*** *for BOD_5 determination (see BOD_5-TT Accessories Set, REF 916925).*

Diluting water and inoculating water:
The manufacture and correct handling of diluting water for BOD_5 determination and inoculating water use is described in detail in the BOD_5-TT-Accessories Set (REF 916925). Please observe the specified data there.

Determination of BOD_5:
Requisite accessories: BOD_5-TT-Accessories Set (REF 916925), graduated cylinders 25 mL, piston pipette 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 approx. 20 °C

Important! Prior to testing, make sure to replace the rubber stoppers of the test tubes with the grey screw caps provided! Incubation of the filled test tubes and subsequent oxygen determination must be performed with screw caps and **not** with rubber stoppers. The rubber stoppers can be disposed of along with the household waste.

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

At each day of analysis ***one*** *control-test tube (diluting water without sample) must be prepared, used as zero value for all test tubes containing sample dilutions. Also when preparing whole* ***test series, only one*** *control-test tube is necessary.*

Fill in a reaction vessel (BOD_5-TT-Accessories Set, REF 916925)
20 mL aerated diluting water, close the vessel and shake strongly for **30 s** to enrich the control solution with oxygen.

Open one **test tube** with reagent **BOD_5-TT R0** and fill to the brim with control solution **without letting air bubbles in**.

Close the test tube with control solution **without letting air bubbles in**, label as “control” and incubate in a water bath or an incubator for **5 days at 20 ± 1 °C** in the dark.

Step 2: Sample dilutions

Depending on the expected BOD_5 of a sample, the most suitable dilution in accordance to the following table must be prepared in a reaction vessel (BOD_5-TT-Accessories Set, REF 916925). If there is no experience regarding the expected BOD_5 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_5 [mg/L O_2]	Dilution	Examples for typical waters	Sample [mL]	Diluting water [mL]
< 5	–	R	20	0
4 – 12	1 + 1	R, B	10	10
10 – 30	1 + 4	R, B	4	16
20 – 60	1 + 9	B	2	18
40 – 120	1 + 19	C	1	19
100 – 300	1 + 49	C, M	0.4	19.6
200 – 600	1 + 99	C, M	0.2	19.8
400 – 1200	1 + 199	M, I	0.1	19.9
800 – 2400	1 + 399	I	0.05	19.95
1000 – 3000	1 + 499	I	0.04	19.96

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

Fill in a reaction vessel (BOD_5-TT-Accessories Set, REF 916925) **sample and aerated diluting water** in accordance to the table above.

Close the reaction vessel and shake strongly for **30 s** to enrich the sample dilution with oxygen.

Open one **test tube** with reagent **BOD_5-TT R0** and fill to the brim with sample dilution **without letting air bubbles in**.

Close the test tube with sample dilution **without letting air bubbles in**, label as “sample” and incubate in a water bath or an incubator for **5 days** at **20 ± 1 °C** in the dark.

Remark: *The reaction vessels added to the BOD_5-TT-Accessories Set can be used for all preparations of any water samples to be tested (control, sample dilutions). Before using for a new preparation, the vessels must be washed thoroughly by using tap water.*

Step 3: Measurement of dissolved oxygen

After 5 days of incubation at 20 ± 1 °C in the dark, the concentration of dissolved oxygen must be determined in ***all*** *incubated test tubes (control and sample dilutions).*

Open test tube, add
2 drops BOD_5-TT R1 and
2 drops BOD_5-TT R2, close **without air bubbles** and shake.
Wait **2 min**.

Open test tube, add
5 drops BOD_5-TT R3, close **without air bubbles**, shake to dissolve the flakes.
Clean outside of test tube and measure.

Measurement:
For *NANOCOLOR*® photometers see manual, test 8-25.

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.

Analytical quality control:
NANOCONTROL BOD_5 (REF 92582)

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