

REF 985 825

Test 8-25

07.14

NANOCOLOR® BOD₅-TT

en

Method:

Tube test for determination of the biochemical oxygen demand in 5 days (BOD₅) in the presence of added nutrients according to 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 EN 25813 - G21 by photometric evaluation of iodine-color.

Range:	2–3000 mg/L O ₂	2–3000 mg/L O ₂
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:Box A: 22 test tubes BOD₅-TTBox B: 1 bottle with 3 mL BOD₅-TT R11 bottle with 6 mL BOD₅-TT R31 bottle with 3 mL BOD₅-TT R2

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. mycotoxins, 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 BOD₅-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 filtered (membrane filtration kit, REF 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 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 BOD₅ 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 BOD₅ determination (see BOD₅-TT - Accessories Set, REF 916 925).

Diluting water and inoculating water:

The manufacture and correct handling of diluting water for BOD₅ determination and inoculating water use is described in detail in the BOD₅-TT-Accessories Set (REF 916 925). Please observe the specified data there.

Determination of BOD₅:

Requisite accessories: BOD₅-TT-Accessories Set (REF 916 925), 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 (BOD₅-TT-Accessories Set, REF 916 925)

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 **BOD₅-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 BOD₅ of a sample, the most suitable dilution in accordance to the following table must be prepared in a reaction vessel (BOD₅-TT-Accessories Set, REF 916 925). If there is no experience regarding the expected BOD₅ of a sample, at least two, preferably three, different dilutions of this sample should be prepared to assure accuracy of the determination. For a 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

M: Raw municipal sewage

I: Heavily polluted industrial waste water

C: Clarified biomass from a sewage plant or mildly polluted industrial waste water

Fill in a reaction vessel (BOD₅-TT-Accessories Set, REF 916 925) **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 **BOD₅-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 BOD₅-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 BOD₅-TT R1 and

2 drops BOD₅-TT R2, close **without air bubbles** and shake.

Wait **2 min**.

Open test tube, add

5 drops BOD₅-TT R3, close **without air bubbles**, shake to dissolve the flakes.

Clean outside of test tube and measure.

Measurement:

For NANOCOLOR® photometers and PF-12 see manual, test 8-25.

Photometers of other manufacturers:

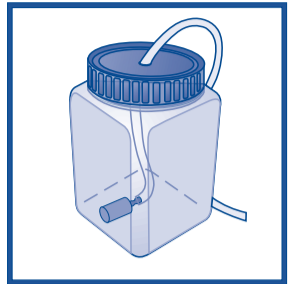
For other photometers check whether measurement of round glass tubes is possible. Verify factor for each type of instrument by measuring standard solutions.

Analytical quality control:

NANOCONTROL BOD₅ (REF 925 82)

Arbeitsschritt 1: Herstellung von Verdünnungswasser
Step 1: Preparation of diluting water
Etape de travail 1 : Préparation de l'eau de dilution
Pase de trabajo 1: Preparación del agua de dilución

- Befüllen des Ansatzbehälters mit ungechlortem Leitungswasser ($\leq 0,01$ mg/L Kupfer, Chlor oder Chloramine), pH 6–8
 Fill the storage vessel with unchlorinated tap water (≤ 0.01 mg/L copper, chlorine or chloramine), pH 6–8
 Remplir le réservoir avec de l'eau de canalisation non chlorée ($\leq 0,01$ mg/L cuivre, chlore ou chloramine), pH 6–8
 Llenar el recipiente para preparación con agua desclorificada ($\leq 0,01$ mg/L cobre, cloro o cloramina), pH 6–8
- Belüftung im Dunkeln bei 20 ± 1 °C für mind. 1 h
 Aerate in the dark at 20 ± 1 °C for at least 1 h
 Le gazage de l'eau de dilution se fait dans l'obscurité à 20 ± 1 °C au moins 1 h
 Airear en la oscuridad a 20 ± 1 °C, durante 1 h

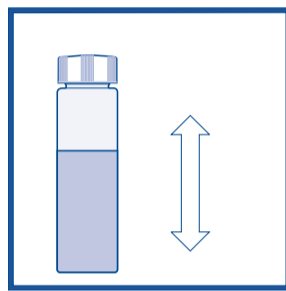


Arbeitsschritt 2a: Kontrollansatz
Step 2a: Control
Etape de travail 2a : Contrôle
Paso de trabajo 2a: Control

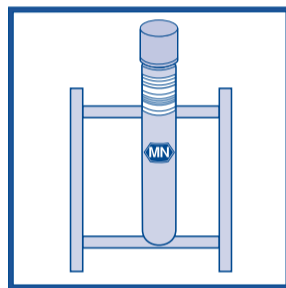
- Befüllen des Reaktionsgefäßes mit 20 mL belüftetem Verdünnungswasser
 Fill the reaction vessel with 20 mL of aerated diluting water
 On remplit un verre à réactif de 20 mL d'eau de dilution aérée
 Llenar el recipiente de reacción con 20 mL de agua de dilución aireada



- Reaktionsgefäß verschließen und 30 s kräftig schütteln
 Close the vessel and shake vigorously for 30 s
 Fermer le récipient et secouer vigoureusement durant 30 s
 Cerrar el recipiente de reacción y agitar energicamente durante 30 s



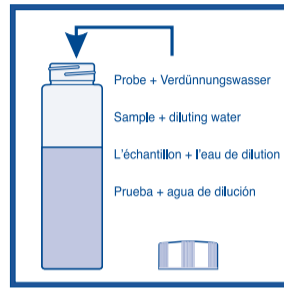
- Eine Rundküvette BSB₅-RKT öffnen und mit Kontrollansatz bis zum Überlaufen luftblasenfrei füllen
 Open one test tube BOD₅-TT and fill to the brim with control solution **without letting air bubbles** in
 Ouvrir une cuvette ronde DBO₅-TCR et remplir de préparation de contrôle **sans bulles d'air** jusqu'à trop-plein
 Abrir un tubo de ensayo DBO₅-TT y llenarla hasta los bordes con preparato de control, **evitando** que se formen **burbujas**



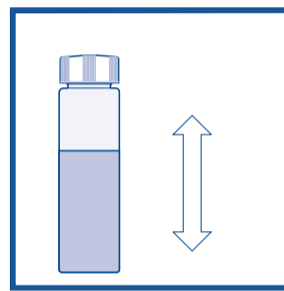
- Rundküvette **luftblasenfrei** verschließen und mit „Kontrolle“ beschriften
 Close the test tube **without letting air bubbles** in and label as „control“
 Fermer la cuvette ronde **sans bulles d'air** et étiqueter avec " contrôle "
 Cerrar el tubo de ensayo **evitando** la formación de **burbujas** y rotularlo con la palabra „control“

Arbeitsschritt 2b: Probenansatz
Step 2b: Sample
Etape de travail 2b : Échantillon
Paso de trabajo 2b: Prueba

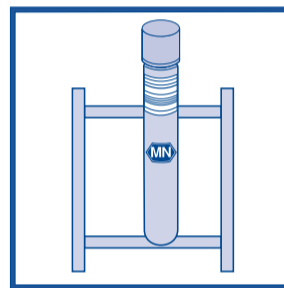
- Befüllen des Reaktionsgefäßes mit Probe und Verdünnungswasser gemäß der Verdünnungstabelle
 Fill in a reaction vessel sample and aerated diluting water in accordance with the dilution table
 On verse dans un verre à réactif l'échantillon et l'eau de dilution aérée conformément au tableau de dilution
 Llenar el recipiente de reacción con prueba y agua de dilución aireada según las tablas de dilución



- Reaktionsgefäß verschließen und 30 s kräftig schütteln
 Close the vessel and shake vigorously for 30 s
 Fermer le récipient et secouer vigoureusement durant 30 s
 Cerrar el recipiente de reacción y agitar energicamente durante 30 s



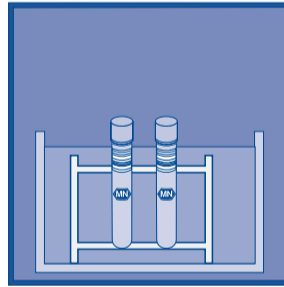
- Eine Rundküvette BSB₅-RKT öffnen und mit Probenansatz bis zum Überlaufen **luftblasenfrei** füllen
 Open one test tube BOD₅-TT and fill to the brim with sample solution **without letting air bubbles** in
 Ouvrir une cuvette ronde DBO₅-TCR et remplir de préparation d'échantillon **sans bulles d'air** jusqu'à trop-plein
 Abrir un tubo de ensayo DBO₅-TT y llenarla hasta los bordes con preparato de control, **evitando** que se formen **burbujas**



- Rundküvette **luftblasenfrei** verschließen und mit „Probe“ beschriften
 Close the test tube **without letting air bubbles** in and label as „sample“
 Fermer la cuvette ronde **sans bulles d'air** et étiqueter avec " échantillon "
 Cerrar el tubo de ensayo **evitando** la formación de **burbujas** y rotularlo con la palabra „prueba“

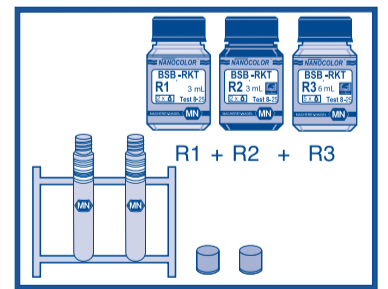
Arbeitsschritt 3: Inkubation
Step 3: Incubation
Etape de travail 3 : Incubation
Paso de trabajo 3: Incubación

- Die angesetzten Rundküvetten im Wasserbad oder Temperierschrank 5 Tage bei 20 ± 1 °C im Dunkeln inkubieren
 Incubate the prepared test tubes in the dark in a water bath or incubator for 5 days at 20 ± 1 °C
 Laisser incuber les cuvettes rondes de préparation dans un bain-marie ou dans une armoire tempérée pendant 5 jours dans l'obscurité à 20 ± 1 °C
 Incubar los tubos de ensayos preparadas en baño de agua o en estufa de incubación, 5 días a 20 ± 1 °C, en la oscuridad



Arbeitsschritt 4: Sauerstoffbestimmung und Auswertung
Step 4: Oxygen determination and evaluation
Etape de travail 4 : Détermination de l'oxygène et évaluation
Paso de trabajo 4: Determinación de oxígeno y valoración

- Nach 5 Tagen Rundküvetten öffnen und Zugabe von 2 Tropfen BSB₅-RKT R1 und 2 Tropfen BSB₅-RKT R2
 After 5 days of incubation, open the test tubes and add 2 drops BOD₅-TT R1 and 2 drops BOD₅-TT R2
 Après une incubation de 5 jours, ouvrir les cuvettes rondes et ajouter 2 gouttes de DBO₅-TCR R1 et 2 gouttes de DBO₅-TCR R2
 Transcurridos 5 días, abrir los tubos de ensayo y añadir 2 gotas de DBO₅-TT R1 y 2 gotas de DBO₅-TT R2



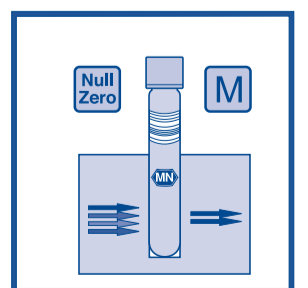
- Rundküvetten **luftblasenfrei** verschließen, kurz schütteln und 2 min warten
 Close test tubes **without air bubbles**, shake briefly and wait for 2 min
 Fermer les cuvettes rondes **sans** laisser de **bulles d'air**, agiter brièvement et attendre 2 min
 Cerrar los tubos de ensayo **evitando** que se formen **burbujas de aire**, agitar brevemente y esperar 2 min

- Rundküvetten wieder öffnen und Zugabe von 5 Tropfen BSB₅-RKT R3
 Open test tubes and add 5 drops BOD₅-TT R3
 Ouvrir les cuvettes rondes et ajouter 5 gouttes de DBO₅-TCR R3
 Volver a abrir los tubos de ensayo y añadir 5 gotas de DBO₅-TT R3

- Küvetten wieder verschließen und kurz schwenken bis sich der Niederschlag aufgelöst hat
 Close test tubes again and shake briefly until the precipitate has dissolved
 Refermer les cuvettes rondes et agiter brièvement jusqu'à ce que le précipité ait été dissout
 Volver a cerrar los tubos de ensayo y disolver el precipitado mediante un movimiento de balanceo breve de los tubos

- NANOCOLOR® Photometer einschalten und Methode 8251 aufrufen
 Switch on the NANOCOLOR® photometer and call up method 8251
 Mettre sous tension le photomètre NANOCOLOR® et appeler la méthode 8251
 Encender el fotómetro NANOCOLOR® y seleccionar el método 8251

- Rundküvette „Kontrolle“ mit Taste messen, anschließend Rundküvette „Probe“ mit Taste messen
 Measure test tube „control“ with key , then measure test tube „sample“ with key
 Mesurer la cuvette ronde de " contrôle " avec la touche , puis mesurer la cuvette ronde " échantillon " avec la touche
 Efectuar la medición en el tubo de ensayo „control“ pulsando la tecla , a continuación el tubo de ensayo „prueba“ pulsando la tecla



- Im Display Anzeige des BSB₅ der Probe in mg/L O₂
 The BOD₅ of the sample is displayed in mg/L O₂
 La valeur DBO₅ mesurée de l'échantillon est indiquée en mg/L O₂
 En el visor del aparato, aparece el DBO₅ de la prueba en mg/L O₂

Simplified determination of BOD₅ in undiluted samples

en

1. Method

Simple determination of the biochemical oxygen demand after 5 days (BOD₅) of **undiluted** samples without using a control in accordance with **DIN EN 1899-2 – H52!** The oxygen enriched, undiluted sample is incubated in test tubes for 5 days at 20 ± 1 °C in the dark. Determination of the dissolved oxygen after 5 days is based on the Winkler procedure, DIN EN 25813 - G21.

2. Procedure

No control is required!

Range: 0.5–7.5 mg/L O₂

Step 1: Sample treatment

For treatment of the samples and as regards possible interferences of a BOD₅ determination, please refer to the remarks in the instructions for use of tube test **NANOCOLOR[®] BOD₅-TT – Test 8-25**.

Step 2: Sample preparation

1. Fill a reaction vessel (BOD₅-TT Accessories Set, REF 916 925) with **20 mL undiluted sample solution**.
2. Close reaction vessel and **shake** vigorously for **30 s** to enrich the oxygen content of the sample.
3. Open a **test tube BOD₅-TT** and fill to the brim with sample **without letting air bubbles in**.
4. Close test tube **without letting air bubbles in**, label and incubate in water bath or incubator for **5 days** at **20 ± 1 °C** in the dark.

Step 3: Oxygen measurement and evaluation

1. After 5 days of incubation perform the **oxygen measurement** in accordance with step 3 of the instructions for use of tube test **NANOCOLOR[®] BOD₅-TT – Test 8-25**
2. **Measurement:** Call up method **8252** and perform measurement. Measure the oxygen concentration of the sample by pressing **key**. The **BOD₅ value of the undiluted** sample is displayed **directly** in **mg/L O₂**.