NANOCOLOR® BOD_5 - TT

Test 8-25

REF 985 825

Tube test

Method: Determination of the biochemical oxygen demand in 5 days (BOD_5) by adding nutrient salts according to the German Standard Method DIN EN 1899-1 - H51 and additional nitrification inhibition with *N*-allylthiourea. Incubation of the samples is performed directly in test tubes. Determination of the dissolved oxygen is carried out similarly to the Winkler method (DIN EN 25813 - G21) by photometric evaluation of the iodine colour.

			Method
Ranges:	2 – 3000 mg/l O_2	(diluted samples)	**8251**
	0.5 – 7.5 mg/l O_2	(non-diluted samples)	**8252**

NANOCOLOR® reagent set: BOD_5-TT (REF 985 825)

Wavelength: **436** nm

Req. accessories: BOD_5-TT accessories set (REF 916 925), piston pipette with disposable tips, graduated cyclinder 25 ml, water bath or incubator (alternative: a dark room with a temperature of about 20 °C)

Procedure:

Preparatory steps

1. 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 is formed, the sample should be homogenised very well or filtrated.

2. Diluting water and inoculating water

The instructions supplied with the BOD_5-TT accessories set (REF 916 925) contain full details about the preparation and handling of the diluting water and inoculating water. Please observe the data given in the instructions.

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

*Per analysis day you have to prepare **one** control tube (water for dilution without sample) which is used as blank value for all samples of that day. For **test series**, too, only **one** control is required.*

Fill a reaction tube (from the BOD_5-TT acessories set) with **20 ml aerated diluting water**, close the tube and shake vigorously 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 **without air bubbles**, label it as “control“ and incubate in a water bath or an incubator for **5 days at 20 ± 1 °C** in the dark.

Step 2: Sample

Depending on the expected BOD_5 of the sample, prepare in a reaction tube (BOD_5-TT accessories set) the most suitable dilution according to the following table.

If there are no experiences regarding the expected BOD_5, at least two, preferably three different dilutions of the 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]	Water for dilution [ml]
< 5	1 : 1	R	20.00	0
4 – 12	1 : 2	R, B	10.00	10.00
10 – 30	1 : 5	R, B	4.00	16.00
20 – 60	1 : 10	B	2.00	18.00
40 – 120	1 : 20	C	1.00	19.00
100 – 300	1 : 50	C, M	0.40	19.60
200 – 600	1 : 100	C, M	0.20	19.80
400 – 1200	1 : 200	M, I	0.10	19.90
800 – 2400	1 : 400	I	0.05	19.95
1000 – 3000	1 : 500	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 water
I = heavily polluted industrial waste water

In a reaction tube (BOD_5-TT accessories set) mix **sample** and **aerated diluting water** in accordance with the table above.

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

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

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

Remark: The reaction tubes included in the BOD_5-TT accessories set can be used for all preparations of water samples to be tested (control, sample dilutions). Before using them for a new preparation, they have to be washed thoroughly with 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 is determined in all incubated test tubes (control and sample dilutions).
Open test tube, add **2 drops of BOD_5-TT R1** and **2 drops of BOD_5-TT R2**, close **without air bubbles** and shake. Wait **2 min**.
Open test tube, add **5 drops of BOD_5-TT R3** close **without air bubbles**, shake to dissolve the flakes. Clean outside of test tube and perform measurement.

Measurement: Call up method **8251**
First press key Null Zero and measure the test tube with the **control**.
Then measure the **sample dilutions** by pressing key M for every sample.
The BOD_5 value of the sample is displayed in mg/l O_2.

Special application: Simplified BOD_5 determination on non-diluted samples

No control necessary!

Fill a rection tube (BOD_5-TT accessories set) with **20 ml non-diluted sample**.
Close the tube and **shake** vigorously for **30 s** to enrich the sample with oxygen.
Open one **test tube** with reagent **BOD_5-TT R0** and fill to the brim with sample solution **without** letting **air bubbles** in.
Close the test tube **without air bubbles**, label and incubate in a water bath or an incubator for **5 days at 20 ± 1 °C in the dark**.
After 5 days of incubation **measure oxygen concentration** in accordance with step 3.
Measurement: Call up method **8252** and perform measurement. The **BOD_5 value of the non-diluted sample** is directly displayed in **mg/lO_2**.

Analytical quality control: *NANOCONTROL* BOD_5 (REF 925 82)