

## 0.5–12 mg/L BOD<sub>5</sub> (5-day biochemical oxygen demand)

**LCK554**

**Scope and application:** For surface water, low-load outflows of municipal or industrial sewage treatment plants with biological purification stage without additional inoculation.



### Test preparation

#### Test storage

Storage temperature: 2–8 °C (35–46 °F)

#### pH/Temperature

The pH of the water sample must be between pH 4–10.

The temperature of the water sample and the dilution water must be between 18–24 °C (64–75 °F).

#### Before starting

**Special note:**

Measuring range for samples, **not diluted**: 0.5–6.0 mg/L BOD<sub>5</sub>

Measuring range for **diluted** samples: 1.0–12.0 mg/L BOD<sub>5</sub>

Samples of surface water that contain **no municipal waste water** are inoculated by means of a simplified procedure **prior to the analysis** refer to [Procedure BOD5 Application](#) on page 4.

The transfer pipettes should be discarded after use. The beakers should be cleaned thoroughly with hot tap water after use, or, if strongly soiled, with a suitable cleansing agent.

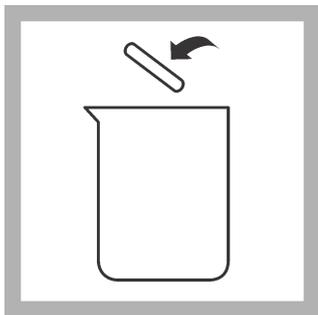
On grounds of quality and reliability, the analysis should be carried out only with original accessories from the manufacturer.

Review safety information and expiration date on the package.

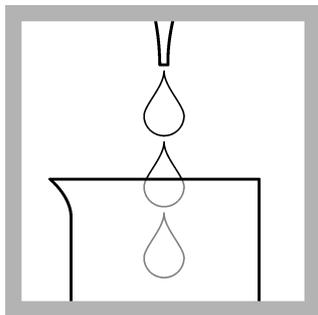
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

## Procedure BOD<sub>5</sub> Sample preparation



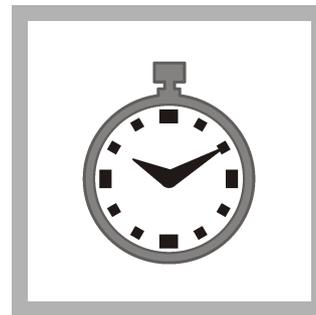
1. Introduce the magnetic stirring bar into a 100 mL beaker.



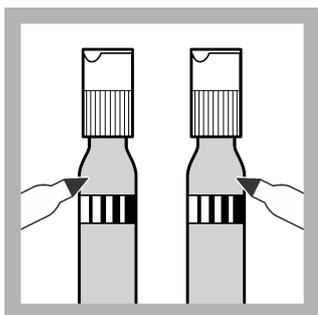
2. a) **Original sample:** Pipet **40 mL wastewater sample** into the beaker.  
2. b) **Diluted sample:** Pipet **20 mL wastewater sample** and **20 mL chlorine-free drinking water** ( $BOD_5 < 0.5 \text{ mg/L}$ ) into the beaker.



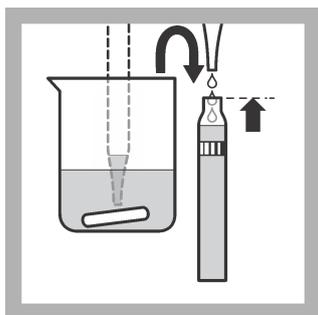
3. Place the beaker on the magnetic stirrer.



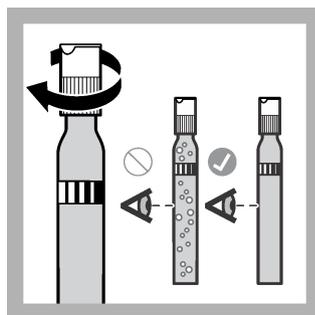
4. Stir for **5 minutes** at 500–750 rpm. This oxygenates the wastewater sample.



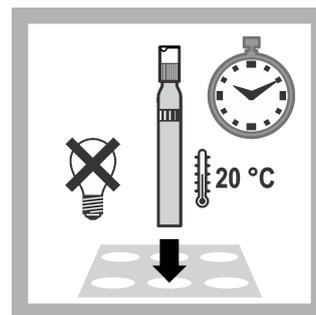
5. **Two sample cuvettes** are needed for each wastewater sample — **a) or b)**.  
Sample **cuvette 1** is measured **directly** and sample **cuvette 2** is measured after **5 days**. It is advisable to **label** the cuvettes: for example **A1 = direct** measurement  
**A5 = measurement after 5 days**.



6. A transfer pipet is used to fill the two sample cuvettes **up to the brim** in sequence with the prepared wastewater sample.

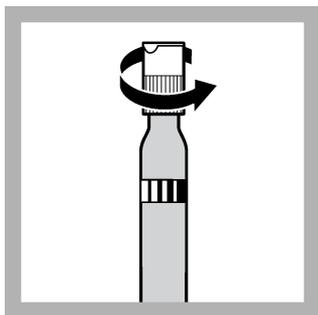


7. Ensure that the sample **cuvette 2** (measurement after **5 days**) is **free of air bubbles** and seal it **immediately**.

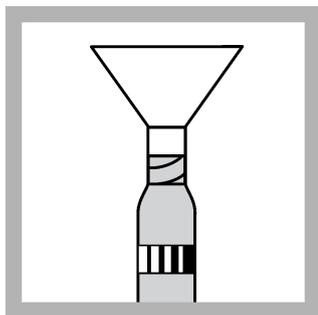


8. Leave it for **5 days** at **20° C** (68° F) in the **dark** in a temperature-controlled cabinet or LT20 dry thermostat.

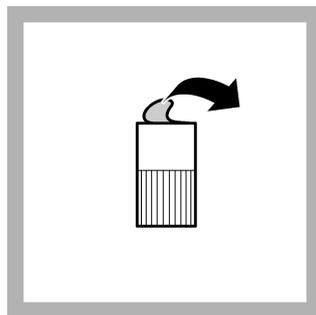
## Procedure BOD<sub>5</sub> Evaluation direct and after 5 days



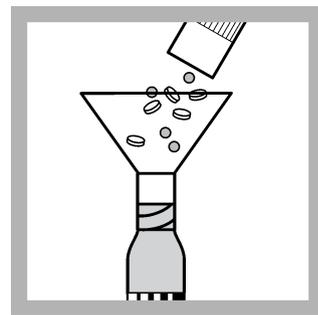
1. Open **cuvette 1**.



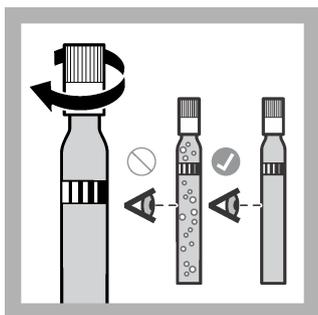
2. Place the funnel on the filled sample **cuvette 1**.



3. Carefully pull the aluminium foil off the DosiCapZip.

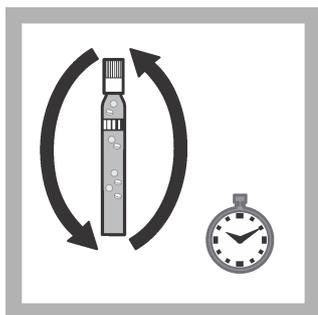


4. Pour the contents of the DosiCapZip (tablets and glass beads) through the funnel into the sample **cuvette 1**.

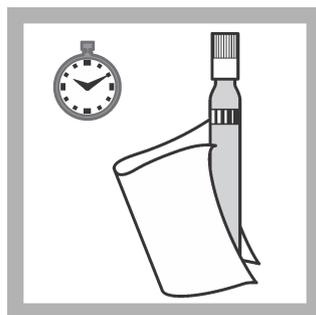


5. Remove the funnel and **immediately** seal the sample **cuvette 1** with **DosiCapZip**, taking care that the cuvette contains **no air bubbles**.

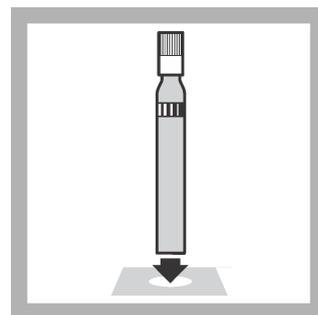
*Note: If the liquid meniscus falls below the cuvette opening when the funnel is removed, make up the volume by adding 2 to 4 glass beads.*



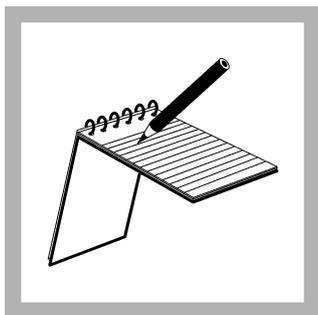
6. Repeatedly invert the sample **cuvette 1** for **3 minutes** until the reagent tablets are completely dissolved.



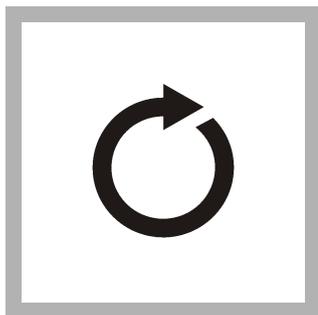
7. After another **3 minutes**, thoroughly clean the outside of the cuvette.



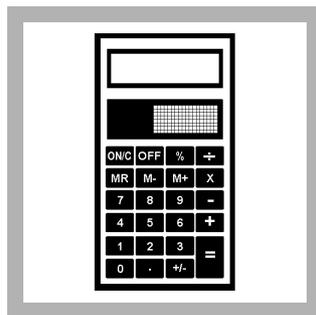
8. Insert the cuvette into the cell holder.  
DR1900: Go to LCK/TNTplus methods. Select the test, push **READ**.



9. Record the result (=A1)!



10. Repeat step 1 to 9 **after 5 days** with sample **cuvette 2** (=A5).



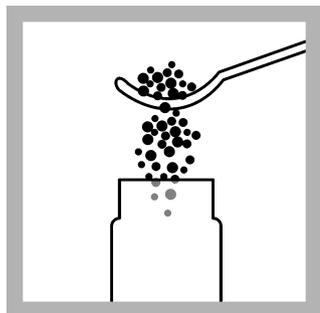
11. Calculation of the BOD<sub>5</sub>-concentration: A1 - A5 = mg/L BOD<sub>5</sub>.  
Diluted samples: The dilution factor must be taken into account when the BOD<sub>5</sub> content is calculated (measurement result x 2).

## Procedure BOD<sub>5</sub> Application

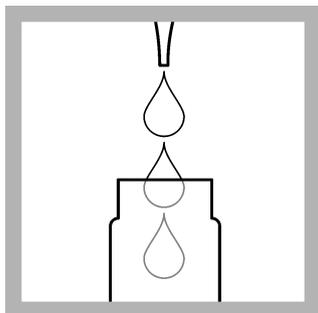
Simplified inoculation procedure for surface water that is only **minimally microbiologically** contaminated. The procedure should be used for surface water that contains no municipal waste water.

**Table 1 Items to collect**

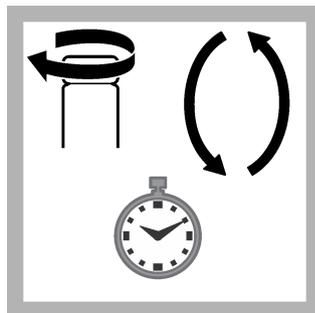
BioKit	LZC555
Reaction tubes with cap	LZP065



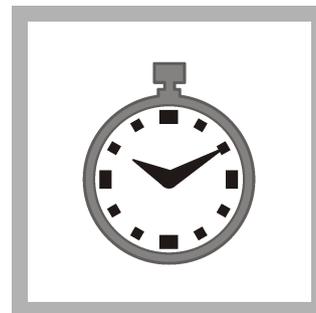
**1. Preparation of the inoculation solution:** Add to reaction tube 1: **1 level dosing spoon** (blue) **inoculation material** (from LZC555)



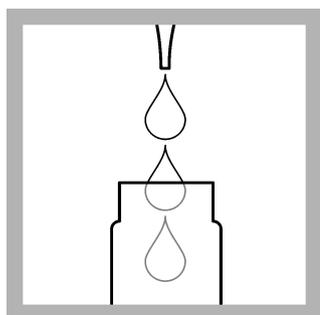
**2. Pipet 10 mL buffer solution** (from LZC555).



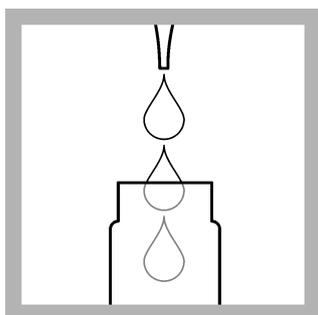
**3. Close and mix thoroughly for 1 minute.**



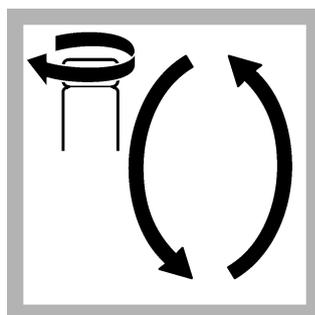
**4. Leave to settle for 20 minutes.**



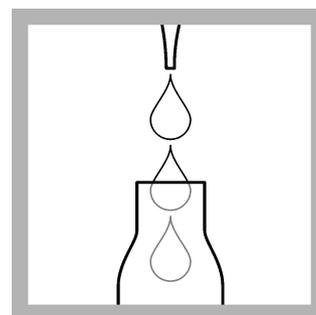
**5. Preparation of the diluted solution:** Pipet into reaction tube 2: **0.2 mL** of solution from reaction tube 1.



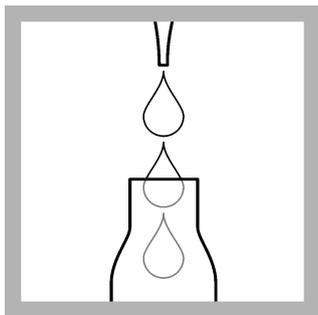
**6. Pipet 10 mL of chlorine-free tap water.**



**7. Close and mix.**



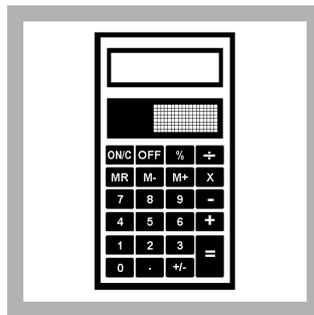
**8. Prepare sample cuvette 1:** Fill sample cuvette 1 with the sample or a diluted (1 : 2) sample, then analyse with [Procedure BOD<sub>5</sub> Evaluation direct and after 5 days](#) on page 3 (1. direct measurement).



**9. Prepare sample cuvette 2:** Pipet **0.2 mL** of the diluted solution from **reaction tube 2** into sample cuvette 2, then top up with sample or diluted (1 : 2) sample.



**10.** Leave to stand for **5 days**, then evaluate with [Procedure BOD5 Evaluation direct and after 5 days](#) on page 3 (2. measurement after 5 days).



**11.** Calculation of the BOD<sub>5</sub> content: Subtract **0.1 mg/L** BOD<sub>5</sub> from the displayed result.

## Interferences

Peroxide compounds, powerful oxidizing agents, high chlorine concentrations and powerful reducing agents cause high-bias or low-bias results and interfere with the biochemical oxidation over 5 days.

Nitrite (NO<sub>2</sub><sup>-</sup>) and Iron (Fe<sup>2+</sup>) can interfere with the reaction if present in concentrations of at least 1 mg/L in the original sample.

The COD content should not exceed 25 mg/L in the original sample or 50 mg/L in the 1 : 2 dilution.

Samples with a high particulate content interfere with the determination—if necessary, carry out the analysis with the supernatant liquid after homogenizing and allowing the sediment to settle out.

The measurement results must be subjected to plausibility checks (dilute and/or spike the water sample). This can be done with LCK554 BOD<sub>5</sub> by means of a multiple determination.

Preserved or frozen samples can **only** be analyzed with this procedure with the application (simplified inoculation procedure).

## Removal of Interferences

Samples that contain algae must be filtered before the analysis (1.2 µm filter; membrane filtration set LCW904; to avoid high-bias results, rinse the filter with distilled water before use).

## Summary of method

Determination of **5-day biochemical oxygen demand** with inhibition of nitrification by **5 mg/L allylthiourea**. The dissolved oxygen is analyzed in an alkaline solution with a pyrocatechol derivative in the presence of Fe<sup>2+</sup>, under which conditions a red dye is formed.

## Table for results

**Special note:**

Measuring range for samples, not diluted: 0.5 - 6.0 mg/L BOD<sub>5</sub>

Measuring range for diluted samples: 1.0 - 12.0 mg/L BOD<sub>5</sub>

Number	Date	Sample and dilution	Result A1 Measuring direct	Result A5 Measuring after 5 days	Result BOD <sub>5</sub> (mg/L) A1 - A5
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					



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