LCK318 Sludge activity (TTC) Screening

DOC312.53.94092

5–200 µg Sludge activity (TTC SA) or 0–500% Residual activity (TTC RA) LCK318

Scope and application: For activated sludge, digested sludge, communal and industrial wastewater.

Test preparation

Test storage

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

Before starting

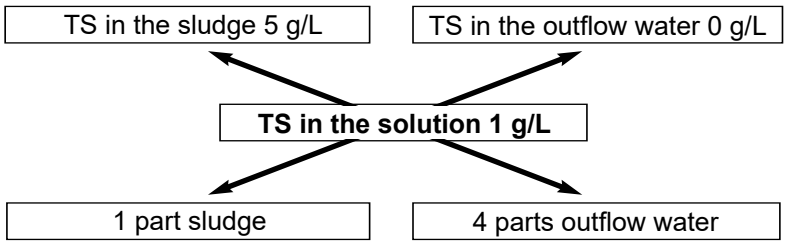
Review safety information and expiration date on the package.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for 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.

Before starting—Visual Evaluation

Application

The degree of stabilization (i.e. the degree of non-digestibility) of sludge can be assessed relatively simply in sewage treatment plants by means of a screening procedure.

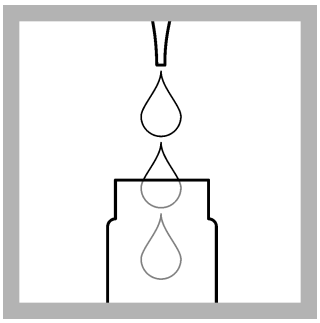
The sludge is diluted with water from the outflow of the final sedimentation tank until its total solids content is about **1 g/L**. The solution can be prepared in line with the following mixing diagram:



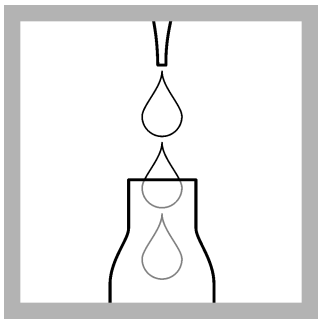
Pipet into the reaction tube:
2 mL sludge + 8 mL outflow water and mix

Procedure—Visual evaluation

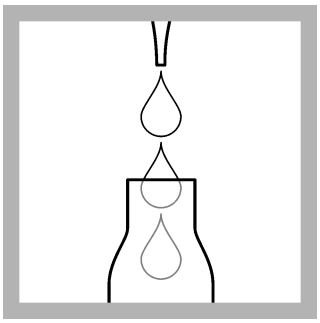
Determine or estimate the total solids in the sludge to **+/-20%**.



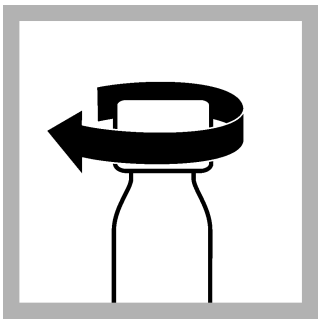
1. Prepare the analysis solution with a TS content of **1 g/L** in a reaction tube (refer to: Before starting—Visual Evaluation on page 1 mixing diagram).



2. Pipet into the cuvette test: **0.6 mL** of buffer **solution A**.



3. Use a transfer pipette to fill the cuvette **to the brim with sample** (make sure that no air bubbles remain in the cuvette).



4. Close the cuvette and keep it in a **dark place** at room temperature (20–25° C (68–77° F)).
Check the cuvette for a red coloration after 30, 45 and 60 minutes.

Evaluation

If, after **one hour, no reddish coloration** of the sludge ”flakes“ is visible, in most cases the sludge has reached the **”technical aerobic stabilization limit“**.

If the sludge is insufficiently stabilized, a clearly discernible red coloration often appears after just **30 minutes**, or after **60 minutes** at most.

Before starting—Method I—Residual activity—TTC RA

Procedure for determining residual activity – TTC RA
Principle
The composition of wastewater can significantly influence the biochemical activity of sludge. The following method is suitable for determining the change in the relative biochemical sludge activity (dehydrogenase activity = DHA) with wastewater samples in less than **2 hours**. The result can be used to assess wastewater, as wastewater can change the biochemical activity of sludge.
Sample preparation
The COD values of the analysed water samples should roughly correspond to the ratio between the values of the inflow and those of the biological stage, otherwise the sample must be diluted with the supernatant water of the aeration tank.

Method I—Sludge activity (TTC) Screening—Residual activity
Concerning the evaluation
The evaluation is carried out in the residual activity measurement mode (TTC RA).The measurement result is expressed as percentage residual activity relative to the reference value. The reference value is documented together with the absolute absorbance.

Evaluating the results

The ”biological“ scatter of the method is **± 10%**. It is advisable to carry out a double determination. Results of less than **80%** residual activity relative to the reference value indicate that the wastewater sample inhibits sludge activity. Further dilutions can be carried out to determine the concentration at which the wastewater sample no longer inhibits sludge activity. Inhibiting substances may be heavy metals (e.g. copper Cu^{2+}) or intermediate substances formed during the biological purification process (e.g. nitrite NO_2^-).

Wastewater samples with a low COD content may seem to inhibit sludge activity, but in this case the observed effects are due to nutrient deficiency. In this case the supernatant liquid of the reference cuvette is diluted in line with the COD load of the sample.

Analysed nutrient-rich wastewater samples may cause an increase in residual activity of more than **120%**. The total solids content of the activated sludge is not taken into account in the relative determination.

Analytical quality assurance

The active sludge must exhibit sufficient activity. Standard substances can be included in the evaluation of activity changes.

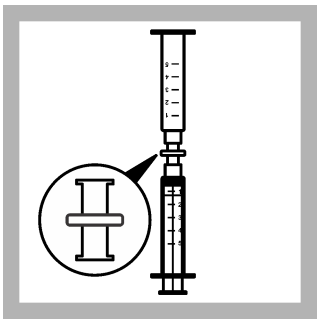
Increases or decreases in residual activity can be checked with these substances to ensure that results are not falsely interpreted.

The active sludge can be tested with a standard inhibitor (e.g. nitrite). Instead of the sample, **2.8 mL** supernatant liquid and **1.0 mL** nitrite standard (1000 mg/L) are used. The residual activity should be **50% ± 20**.

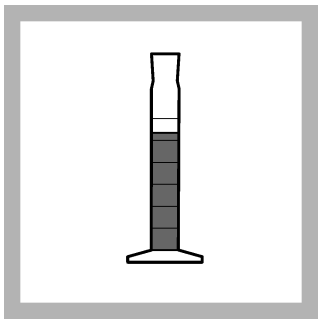
Note

If the sludge is to be used for a long period of time, it is advisable to use the Dilution Water Set LZC901. Transfer about **500 mL** activated sludge from the aeration tank to the vessel and aerate it.

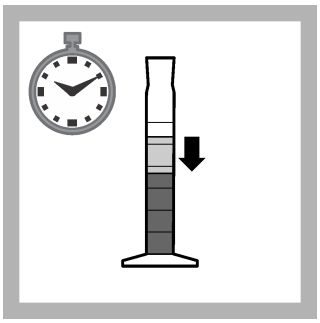
Procedure—Method I—Determining residual activity—TTC RA



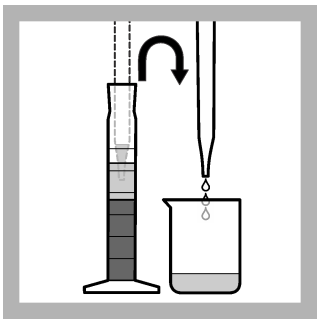
1. Set-up: Use the adapter to join syringe and syringe extension.



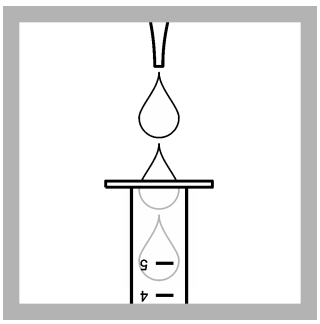
2. Fill a 25 mL measuring cylinder with activated sludge.



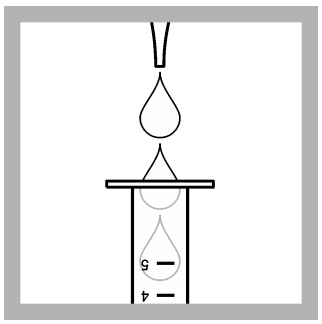
3. Allow the activated sludge to settle for **30 minutes**.



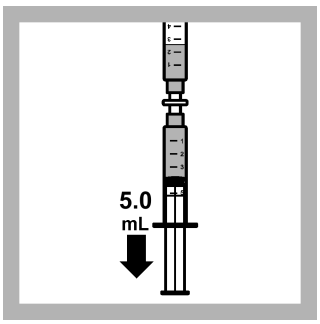
4. Use a transfer pipette to transfer the supernatant liquid into a glass beaker.



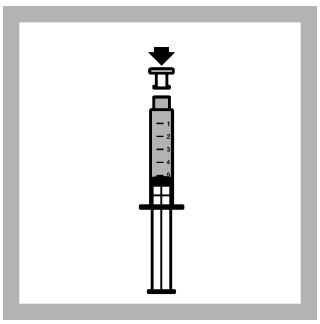
5. Pipet into the **first** syringe extension (**Reference value**): **0.5 mL** activated sludge suspension, **3.8 mL** supernatant liquid and **0.5 mL** buffer **solution A**.



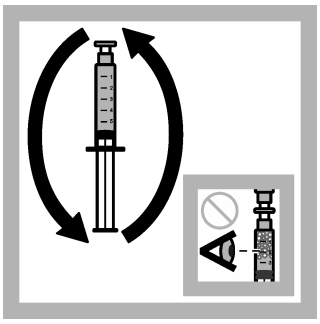
6. Pipet into the **second** syringe extension (**Sample value**): **0.5 mL** activated sludge suspension, **3.8 mL** sample and **0.5 mL** buffer **solution A** .



7. Draw in until plunger is below the **5.0 mL mark**. Transfer contents **free of air bubbles** into the syringe, remove the syringe extension.

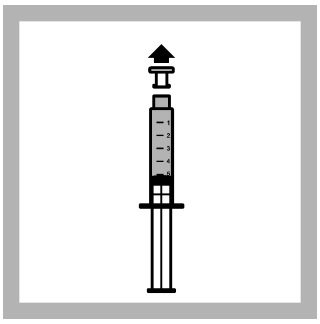


8. Close the syringe.

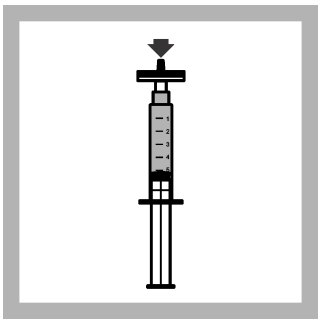


9. Invert a few times and place in the reaction tube stand.

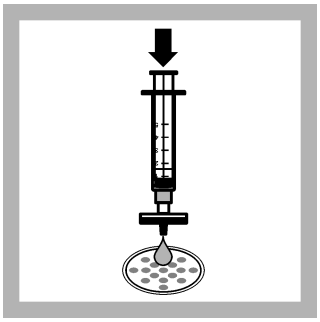
10. Incubate for **1 hour** at constant room temperature (20–25° C (68–77° F)).



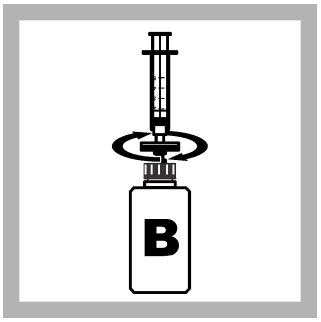
11. Remove cap.



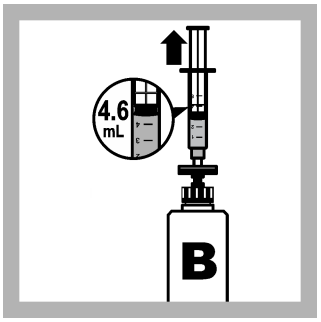
12. Screw on the membrane filter (LCW904).



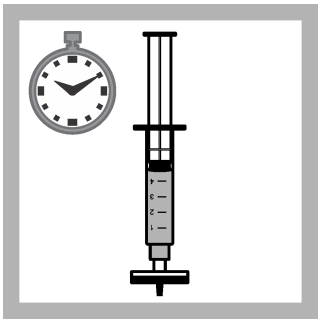
13. Filter the incubated sample, discard the filtrate and wipe off any water drops adhering to the membrane filter.



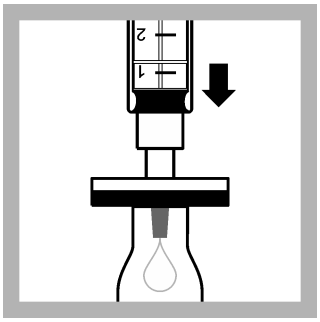
14. Screw the adapter **loosely** onto the bottle containing **solution B** and remove the cap.
Screw the syringe with the membrane filter onto the bottle.



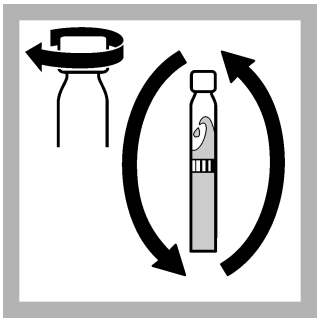
15. Slowly draw **solution B** through the membrane filter into the syringe until it reaches the **4.6 mL** mark.
Close the bottle containing solution B **securely** after use.



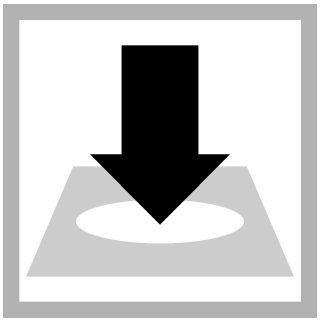
16. Leave to stand for **10 minutes**.



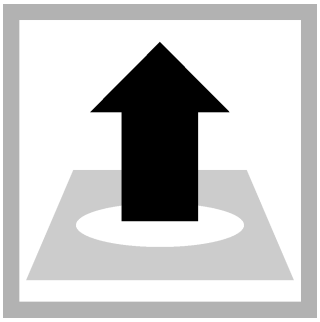
17. Filter the contents of the syringe carefully into the **sample** cuvette.



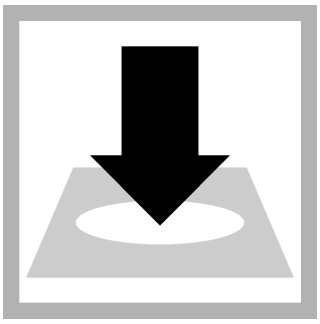
18. Close the cuvette and invert a few times.



19. Insert the **reference** cuvette into the cell holder.
DR1900: Go to LCK/TNTplus methods.
Select the test, push: **READ 1**.



20. Remove the reference cuvette.



21. Insert the **sample** cuvette into the cell holder. DR1900: Push: **READ 2**.

Before starting—Method II—Sludge activity (A_S)—TTC SA

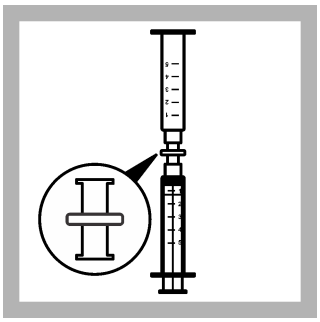
Method II—Sludge activity (TTC) Screening

The method is suitable for determining the enzymatic activity of the activated sludge. The active sludge can be taken **directly** from the aeration tank for analysis. The total solids content [1)] (TS) of the active sludge should not exceed **5 g/L**, otherwise it must be diluted. The origin of the sludge should be specified together with the result, as the result may be influenced by the type of sludge. Floating and bulking sludge are not suitable for this routine analysis.

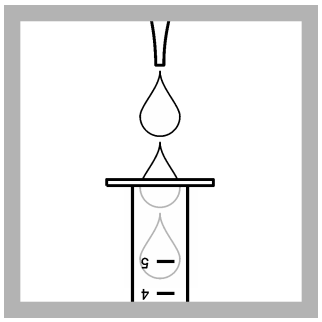
The analyses can be carried out at room temperature (20–25° C (68–77° F)). A constant incubation temperature should be selected for comparative measurements over time (e.g. hydrographs).

[1)] The total solids content is determined at 105° C (221° F). The total organic solids (oTS) can also be used as a reference variable.

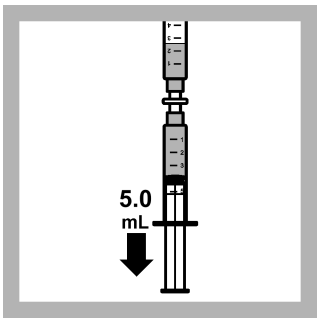
Procedure—Method II—Determining sludge activity (A_S)—TTC SA



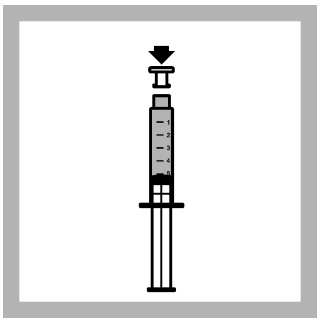
1. Set-up: Use the adapter to join syringe and syringe extension.



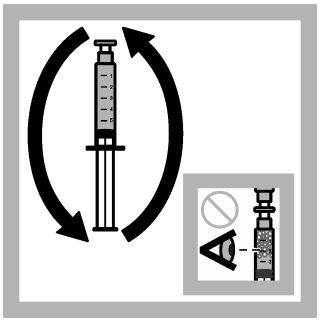
2. Pipet into the syringe extension:
4.3 mL activated sludge suspension and **0.5 mL** buffer **solution A**.



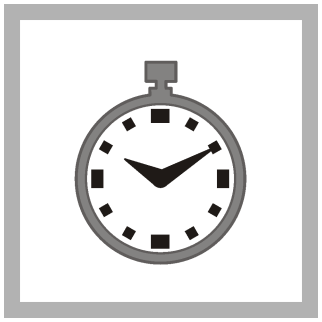
3. Draw in until plunger is below the **5.0 mL mark**. Transfer contents **free of air bubbles** into the syringe, remove the syringe extension.



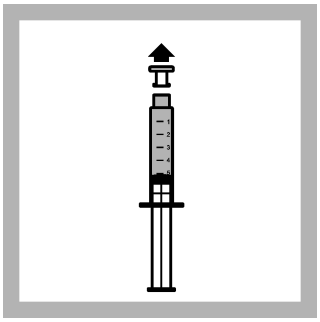
4. Close the syringe.



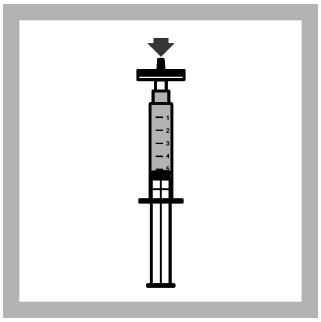
5. Invert a few times and place in the reaction tube stand.



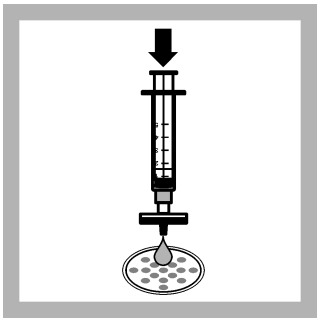
6. Incubate for **1 hour** at constant room temperature (20–25° C (68–77° F)).



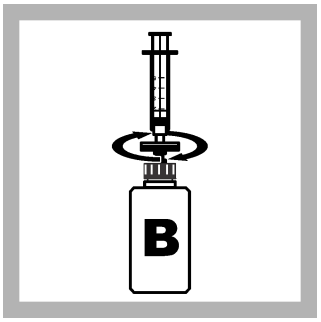
7. Remove cap.



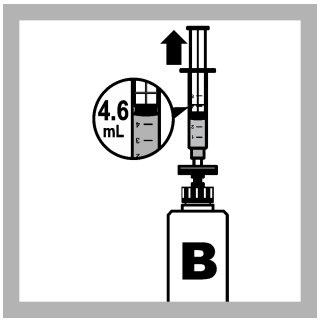
8. Screw on the membrane filter (LCW904).



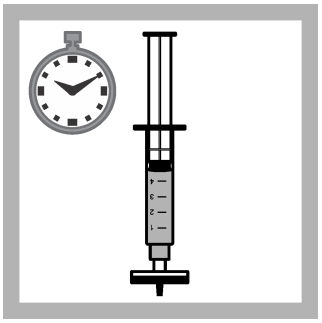
9. Filter slowly the incubated sample, discard the filtrate and wipe off any water drops adhering to the membrane filter.



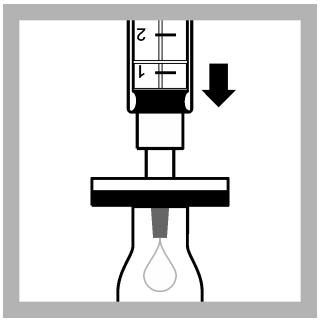
10. Screw the adapter **loosely** onto the bottle containing **solution B** and remove the cap.
Screw the syringe with the membrane filter onto the bottle.



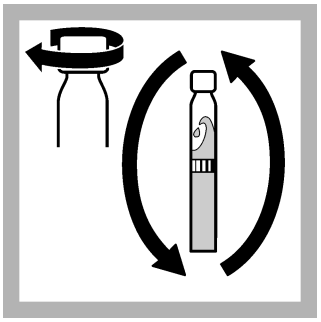
11. Slowly draw **solution B** through the membrane filter into the syringe until it reaches the **4.6 mL** mark.



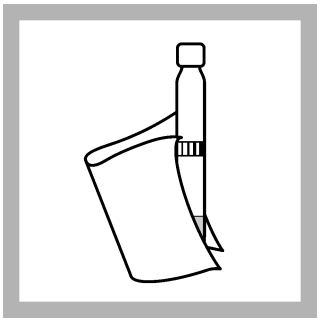
12. Leave to stand for **10 minutes**.



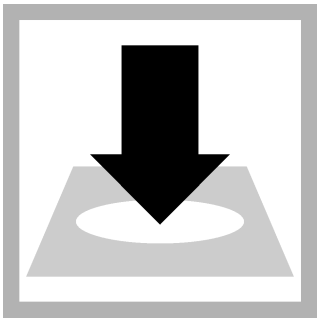
13. Filter the contents of the syringe **carefully** into the sample cuvette.
Close the bottle containing solution B **securely** after use.



14. Close the cuvette and invert a few times.



15. Thoroughly clean the outside of the cuvette and evaluate.



16. Insert the cuvette into the cell holder.

Concerning the evaluation—Method II—Sludge activity—TTC

Concerning the evaluation

In the sludge activity measurement mode (TTC SA) the result is shown in µg formazan.

This result must be related to the total solids.

Calculating the biochemical activity A_S :

Concentration of formazan (µg): C1 = Measurement result

Concentration of activated sludge (mg): C2 = V x TS ; V = 4.3 mL

$$\text{Sludge activity } A_S = \frac{\text{µg formazan}}{\text{mg sludge total solids}} = \frac{\mathbf{C1}}{\mathbf{C2}}$$

TS = Total solids content (g/L)

V = Volume of active sludge (mL)

As = Activity of the sludge expressed in µg formazan, represented by 1 mg sludge total solids

Summary of method

Determination of sludge activity respectively residual activity (activated sludge, digested sludge, etc.) with 2,3,5–triphenyltetrazolium chloride (TTC) on the basis of dehydrogenase activity. TTC is converted to red formazan by dehydrogenases.

The water-insoluble formazan is extracted with ethanol and determined photometrically.

HACH LANGE GMBH
Willstätterstraße 11
D-40549 Düsseldorf
Tel. +49 (0) 2 11 52 88-0
Fax +49 (0) 2 11 52 88-143
info-de@hach.com
www.hach.com

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