

Sludge activity (TTC) Screening

Principle

Determination of sludge activity resp. 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.

Range of Application

Activated sludge, digested sludge, communal and industrial wastewater

Storage Information

The test reagents are stable at +2 to +8°C up to the expiry date given on the package.

Safety Advice

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

CADAS 100 (≥ LPG 210)

If this test is not already stored in your instrument please ask your HACH LANGE Agency for programming instructions.

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for Method I or II

Draw in until plunger is below the 5 mL mark.

Fill syringe(s): Transfer contents to the syringes.

C

Screw on adapter loosely

Screw on filter: Screw syringe(s) with filter onto solution B.

B **F**

Cap

Incubation: Close syringe(s) and incubate as described in working instructions.

D

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

Syringe extension
Adapter
Syringe

A

Pipette: Fill syringe extension(s) as described in Method I or II.

B

1. Filtration: After incubation: screw on a membrane filter and filter slowly (discard filtrate).

Membrane filter
Filter

E

2. Filtration: Slowly draw in 4.6 mL solution B up to the mark. (Close bottle carefully after use).

Draw in slowly

B **G**

Evaluation: After 10 min filter into the sample cuvette and evaluate.

Filter

H

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Edition 02/07

Evaluation TTC SA

1. Press any key.
2. Check program control number: __ : **44**
3. Select test with ↑ or ↓ key.
Control number must be **1*** (see below).
4. Insert sample cuvette.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Press any key.
2. Check program control number: __ : **44**
3. Select test with ↑ or ↓ key.
Control number must be **1*** (see below).
4. Insert reference cuvette. Display: Stand.
5. Insert sample cuvette

If more than one sample is to be measured start the next evaluation at point 5.

Parameter	Display	Meas. range
Sludge activity (TTC SA)	TTCSA LCK 318 1*	5 – 200 µg
Sludge activity (TTC RA)	TTCRA LCK 318 1*	0 – 500 %

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Evaluation TTC SA

1. Insert filter **480 nm**.
2. Select »Dr. Lange« mode.
3. Select test number (see below).
4. Control number must be **3**.
5. Insert sample cuvette and press green key.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Insert filter **480 nm**.
2. Select »Dr. Lange« mode.
3. Select test number (see below).
4. Control number must be **3**.
5. Insert reference cuvette and press green key.
6. Insert sample cuvette and press green key.

If more than one sample is to be measured start the next evaluation at point 6.

Parameter	Test-No.	Meas. range
Sludge activity (TTC SA)	318	5 – 200 µg
Sludge activity (TTC RA)	318	0 – 500 %

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Evaluation TTC SA

1. Select »Barcode Programs«.
2. Select test number (see below).
3. Control number must be **3**.
4. Insert sample cuvette and press »Read«.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Select »Barcode Programs«.
2. Select test number (see below).
3. Control number must be **3**.
4. Insert reference cuvette and press »Read 1«.
5. Insert sample cuvette and press »Read 2«.

If more than one sample is to be measured start the next evaluation at point 5.

Parameter	Test-No.	Meas. range
Sludge activity (TTC SA)	318	5 – 200 µg
Sludge activity (TTC RA)	318	0 – 500 %

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Evaluation TTC SA

1. Insert program filter **470 nm**.
2. Press "Tests" key until display (see below) appears.
3. Control number must be **3**.
4. Insert blank-value cuvette – **LCW 919** (distilled water) and press "Null" (zero) key.
5. Insert sample cuvette and press "Ergebnis" (result) key.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Insert program filter **470 nm**.
2. Press "Funktion" key until display: Standard appears.
3. Press "Null" (zero) key.
4. Press ↑, insert **100** as the value for the standard-concentration. Press ↑ again.
5. Insert reference cuvette and press "Ergebnis" (result) key.
6. Insert sample cuvette and press "Ergebnis" (result) key.

If more than one sample is to be measured start the next evaluation at point 6.

Parameter	Display	Meas. range
Sludge activity (TTC SA)	Test __	5 – 200 µg
Sludge activity (TTC RA)	Test __	0 – 500 %

**Sludge activity (TTC)** Screening

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Evaluation TTC SA

1. Insert sample cuvette.
2. Select evaluation mode TTC SA.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Insert reference cuvette.
2. Select evaluation mode TTC RA.
3. Insert sample cuvette.

If more than one sample is to be measured, set the evaluation mode to permanent.

Parameter	Meas. range
Sludge activity (TTC SA)	5 – 200 µg
Sludge activity (TTC RA)	0 – 500 %

Sludge activity (TTC) Screening

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Evaluation TTC SA

1. Check program control number:
 __ : 48 (CADAS 200)
 __ : 48 (ISIS 6000) ⇒ Select »CUVETTE TEST« mode.
2. Select test number (see below).
3. Control number must be 3.
4. Insert sample cuvette and press green key.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Check program control number:
 __ : 48 (CADAS 200)
 __ : 48 (ISIS 6000) ⇒ Select »CUVETTE TEST« mode.
2. Select test number (see below).
3. Control number must be 3.
4. Insert reference cuvette and press green key.
5. Insert sample cuvette and press green key.

If more than one sample is to be measured start the next evaluation at point 5.

Parameter	Test-No.	Meas. range
Sludge activity (TTC SA)	318	5 – 200 µg
Sludge activity (TTC RA)	318	0 – 500 %

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Evaluation TTC SA

1. Select »TEST« mode.
2. Select symbol (see below).
3. Control number must be 3.
4. Insert blank-value cuvette – **LCW 919** (distilled water) and press "NULL" (zero) key.
5. Insert sample cuvette and press "MESS" (measure) key.

The result is displayed in µg formazan.

Evaluation TTC RA

1. Select »TEST« mode.
2. Select symbol (see below).
3. Control number must be 3.
4. Close cuvette compartment – without cuvette – and press "NULL" (zero) key.
5. Insert reference cuvette and press "MESS" (measure) key.
6. Insert sample cuvette and press "MESS" (measure) key.

If more than one sample is to be measured start the next evaluation at point 5.

Parameter	Symbol	Meas. range
Sludge activity (TTC SA)	318 S	5 – 200 µg
Sludge activity (TTC RA)	318 R	0 – 500 %

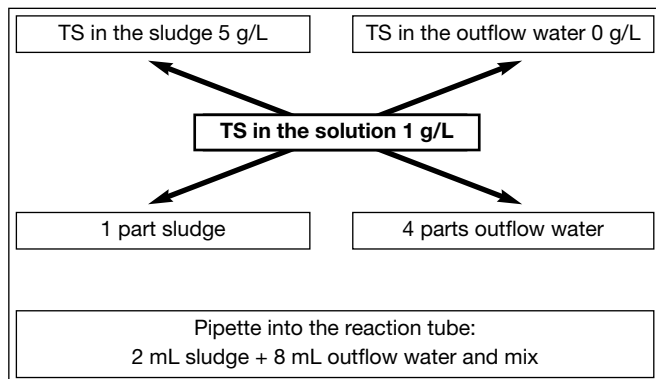
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Application

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:



Applies to all types of photometer

Sludge activity (TTC) Screening

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Procedure Sludge activity (A_S) – TTC SA –

Principle

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 content¹⁾ (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). 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.
The total organic solids (oTS) can also be used as a reference variable.

Pipette into the syringe extension (diagrams A + B)	
Activated sludge sample	4.3 mL
Buffer solution A (LCK 318 A)	0.5 mL
Transfer contents free of air bubbles into the syringe, remove the syringe extension and close the syringe. Invert a few times and place in the reaction tube stand. Incubate for 1 hour at constant room temperature (20 – 25°C). Remove cap and screw on the membrane filter (LCW 904) (diagrams C + D).	
Filter the incubated sample, discard the filtrate and wipe off any water drops adhering to the membrane filter (diagram E).	
Screw the adapter loosely onto the bottle containing solution B (LCK 318 B) and remove the cap. Screw the syringe with the membrane filter onto the bottle. Slowly draw solution B (LCK 318 B) through the membrane filter into the syringe until it reaches the 4.6 mL mark. Leave to stand for 10 min (diagrams F + G).	
Filter the contents of the syringe carefully into the sample cuvette. Close the cuvette, invert a few times and evaluate. Close the bottle containing solution B (LCK 318 B) securely after use (diagram H).	

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Procedure

Determine or estimate the total solids in the sludge to +/-20%	
Prepare the analysis solution with a TS content of 1 g/L in a reaction tube (see <i>mixing diagram</i>)	
Pipette into the cuvette test	
Buffer solution A (LCK 318 A)	0.6 mL
Use a transfer pipette to fill the cuvette to the brim with sample (ensure that no air bubbles remain in the cuvette). Close the cuvette and keep it in a dark place at room temperature (20 -25°C).	
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.

Applies to all types of photometer

Sludge activity (TTC) Screening

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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

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

TS = Total solids content (g/L)

V = Volume of active sludge (mL)

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

Applies to all types of photometer

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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.

Applies to all types of photometer

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Allow the activated sludge to settle in a 25 mL measuring cylinder for **30 min**.

After **30 min** use a transfer pipette to transfer the supernatant liquid into a glass beaker.

Pipette into the first syringe extension (**diagrams A + B**)

Reference cuvette solution:

Activated sludge suspension	0.5 mL
Supernatant liquid	3.8 mL
Buffer solution A (LCK 318 A)	0.5 mL

Pipette into the second syringe extension (**diagrams A + B**)

Sample cuvette solution:

Activated sludge suspension	0.5 mL
Sample	3.8 mL
Buffer solution A (LCK 318 A)	0.5 mL

Transfer contents **free of air bubbles** into the syringe, remove the syringe extension and close the syringe. Invert a few times and place in the reaction tube stand. Incubate for **1 hour** at constant room temperature (20 – 25°C). Remove cap and screw on the membrane filter (LCW 904) (**diagrams C + D**).

Filter the incubated sample, discard the filtrate and wipe off any water drops adhering to the membrane filter (**diagram E**).

Screw the adapter **loosely** onto the bottle containing solution B (LCK 318 B) and remove the cap. Screw the syringe with the membrane filter onto the bottle. **Slowly** draw solution B (LCK 318 B) through the membrane filter into the syringe until it reaches the **4.6 mL** mark. Leave to stand for **10 min** (**diagrams F + G**).

Filter the contents of the syringe **carefully** into the sample cuvette. Close the cuvette, invert a few times and evaluate. Close the bottle containing solution B (LCK 318 B) securely after use (**diagram H**).

Applies to all types of photometer

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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 $\pm 10\%$. 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 $50\% \pm 20$.

Note

If the sludge is to be used for a long period of time, it is advisable to use the **Dilution Water Set LZC 901**. Transfer about **500 mL** activated sludge from the aeration tank to the vessel and aerate it.

LP2W	02/07
TTC SA • $F_1 = 0 \cdot F_2 = 83 \cdot K = -1$	
TTC RA • Std-Conc.: 100	
CADAS 30/30S/50/50S	02/07
TTC SA • $\lambda: 478 \text{ nm} \cdot \text{Pro.: } 1 \cdot F_1 = 0 \cdot F_2 = 79 \cdot K = -3.800$	
TTC RA • $\lambda: 478 \text{ nm} \cdot \text{Pro.: } 3 \cdot F_1 = 0 \cdot F_2 = 99.91 \cdot K = 0$	
ISIS 6000/9000	02/07
TTC SA • $\lambda: 500 \text{ nm} \cdot \text{Pro.: } 1 \cdot F_1 = 0 \cdot F_2 = 85 \cdot K = -4.483$	
TTC RA • $\lambda: 500 \text{ nm} \cdot \text{Pro.: } 3 \cdot F_1 = 0 \cdot F_2 = 99.96 \cdot K = 0$	
CADAS 100 / \geq LPG 210	02/07
TTC SA • $\lambda: 478 \text{ nm} \cdot F_1 = 79 \cdot K = -1.001$	
TTC RA • $\lambda: 478 \text{ nm} \cdot F_1 = 99.91 \cdot K = 0$	
CADAS 200 Barcode / Basis / Combimodule	02/07
TTC SA • $E1W1 \cdot C1 = E1 \cdot F1 - F2 \cdot W1 = 478 \text{ nm} \cdot F1 = 77 \cdot F2 = 4.386$	
TTC RA • $E1W1.(M.E2W1) \cdot C1 = E2 \cdot F1 / E1 \cdot W1 = 478 \text{ nm}$ $F1 = 99.98 \cdot F2 = 0$	