REF 985890

Test 8-90 04.23

NANOCOLOR® TTC / Sludge activity 150

Method:

Determination of the biochemical activity of sludge (activated sludge, digested sludge etc.) by means of the dehydrogenase activity using 2,3,5-triphenyltetrazoliumchloride (TTC). Colourless TTC is converted into red triphenylformazane (TPF) by dehydrogenases. The formed, water-insoluble TPF is dissolved in ethanol and is photometrically determined.

The rapid test enables:

1. the determination of the biochemical activity (A_S) of sludge in terms of the µg TPF/mg of the dry sludge mass (**method 8901**)
2. the characterization of the effect of waste water and waste water compounds on sludge (stimulation or inhibition of the dehydrogenase activity DHA in percentage) (**method 8902**)
3. a rapid, visual valuation of the degree of stabilisation of sludge based on a simple screening method (**method 8903**)

Content of reagent set:

Range:	**5 – 150 µg TPF (triphenylformazane) (method 8901)**	**0.050 – 2.300 E (method 8902)**
Factor:	**0071**.	–
Wavelength (HW = 5 – 12 nm):	**470 nm**	
Reaction time:	**30 – 120 min**	
Reaction temperature:	**20 – 25 °C**	

Box A: 20 empty test tubes TTC 150
3 syringes 5 mL
Box B: 1 bottle with 30 mL TTC 150 R1
2 bottles with 60 mL TTC 150 R2
2 syringe tubes 5 mL
1 reaction vessel 40 mL
1 screw plug with suction pipe
3 Luer-Lock seal plugs female
2 Luer-Lock connecting adaptors female/female
1 Luer-Lock seal plug male
Box C: 21 membran filters ∅ 1.2 µm

Hazard warning:

Reagent R2 contains ethanol 90 – 98 %.
For further information ask for a safety data sheet.

Interferences:

The triphenylformazane (TPF) which is formed is very light sensitive and hence the test samples should be incubated in the dark. Oxygen inhibits the TTC reduction; for this reason the test samples in the syringe tubes should have a constant volume and no oxygen should be enclosed within them. The dehydrogenase activity is not just hindered by oxygen but also by NO_3^-, Fe^{3+} and NO_2^-. A stimulating effect have P_i, Fe^{2+}, SO_4^{2-}, Cl^- and Mn(IV).

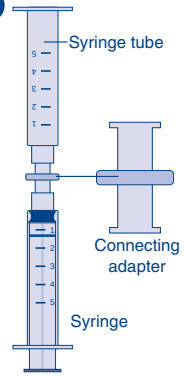
Storage:

Store the test kit in a cool (2 – 8 °C) and dry place.

Practical tips:

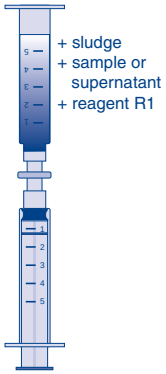
1. The result can be influenced by the type and origin of the sludge amongst other things. Hence the origins of the sludge should at least be added to the result. Scum or bulking sludge are not appropriate for routine tests in accordance with method **8902**. The processes which have been listed are, however, appropriate for the test of digested sludge.
2. The tests can be carried out at room temperature. Execution at constant, defined incubation temperatures is recommended to order to achieve better comparability of the results of series tests such as hydrograph curves. The incubation of the test samples should always be carried out in the dark.
3. **Use of a standard when testing waste waters (method 8902):** The activated sludge must be sufficiently active. This is best carried out by using a nitrite solution (1000 mg/L) as standard inhibitor. **3.0 mL** of the supernatant and **1.0 mL** of nitrite solution are used instead of the sample. The inhibition of the dehydrogenase activity of this test sample should be between 50 ± 20 %.
4. The dry sludge mass DSM is determined by being dried at 105 °C.

1



Connect the syringe tube with the syringe by using the Luer-Lock connecting adapter.

2

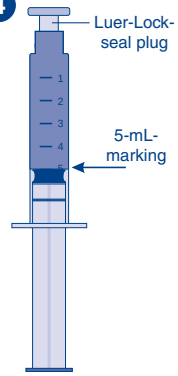


Fill the syringe tube(s) in accordance with the test instructions.

3

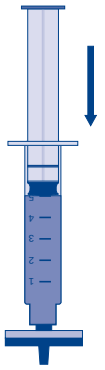
Transfer the content of the syringe tube into the syringe.

4



Close the syringe with the seal plug **without air bubbles** an incubate it in accordance with the instructions.

5

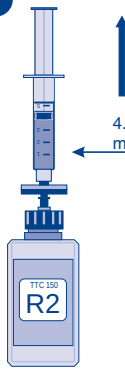


Filtration:
Screw on the disposable filter an slowly filter the solution (discard the filtrate).

6

Preparation of the extraction:
Screw on the disposable filter onto the bottle with R2.

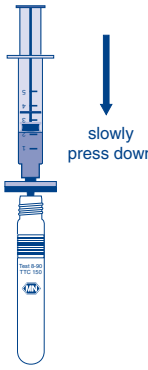
7



Extraktion:
Slowly siphon off reagent R2 up to the 4.6-mL-marking.

(seal the bottle with R2 once more)

8



Evaluation:
After 10 min filter the solution into the empty test tube an measure.

Method 8901: Determination of the biochemical activity of sludge (A_S)

The process serves to determine the biochemical activity of activated sludge. For the purposes of testing the activated sludge which must be tested can be **directly** removed from the activated sludge basin. The dry sludge mass (DSM) of the activated sludge may not exceed a level of **5 g/L.**

Procedure:

	Working step	Picture
1.	Connect a syringe 5 mL with a syringe tube by using a Luer-Lock connceting adapter female/female.	**1**
2.	Add into the syringe tube **4.5 mL activated sludge sample** and **0.5 mL** reagent **R1**.	**2**
3.	The content of the syringe tube is subsequently transferred into the syringe 5 mL **without air bubbles**. Seal syringe with a Luer-Lock seal plug (female) without air bubbles, shake and place in a test tube rack. Incubation for **1 h at room temperature in the dark.**	**3 + 4**
4.	Remove the seal plug and screw on the membrane filter.	**5**
5.	Filter the test sample and discard the filtrate. Wipe off the adherent liquid drops on the membrane filter with a disposable cloth.	**5**
6.	Screw on the screw plug with the suction pipe **loosely** to the bottle with the reagent R2. Following this screw on the syringe with the membrane filter onto the bottle.	**6**
7.	**Slowly** draw off the reagent R2 via the membrane filter into the syringe up to the marking of **4.6 mL.** Incubation for **10 min at room temperature in the dark**.	**7**
8.	Following this press the contents of the syringes carefully into an empty test tube, seal the test tube, clean it on the outside and call up **method 8901**.	**8**

Measurement:
For *NANOCOLOR®* photometers see manual, test 8-90.

Calculation of the biochemical activity A_S:
Activated sludge concentration C_S in the test sample in mg = V x DSM

Biochemical activity A_S [µg Formazan / mg dry sludge mass] ≈ C_{TPF} / C_S

V = Volume of the activated sludge sample in ml
DSM = Dry sludge mass of the activated sludge in g/L when dried at 105 °C
C_{TPF} = Concentration of triphenylformazane in the test sample in µg

Evaluation sheet
"Determination of the biochemical activity AS of sludge" (method 8901)

Date	Sample description	C_{TPF} [µg TPF]	C_S [mg]	Biochemical activity $A_S \approx C_{TPF}/C_S$ [µg formazane / mg dry sludge mass]

Method 8902: Determination of the relative change of the dehydrogenase activity DHA (biochemical sludge activity) due to waste water and waste water compounds

The composition of waste water can considerably influence the activity of the sludge. With the aid of the following procedure the relative change of the dehydrogenase activity (DHA) which is caused by waste water or individual waster water substances can be evaluated within 2 h. In this way statements can be made with respect to the compatibility of the waste water which has been passed into it in terms of the existing biology.

Procedure:

Notice: *The COD burden of the sample which must be tested should be within the concentration range of waste water which flows to the biology ("Influent biology").*

Working step		Picture
1.	Sediment the activated sludge in a suitable laboratory vessel (e.g. measurement cylinder 25 mL) for **30 min**.	
2.	Following this transfer the supernatant with a transfer pipette into a beaker.	
3.	Preparation of two syringes 5 mL which are respectively connected with a syringe tube by using Luer-Lock connecting adapters female/female.	1
4.	**Reference value:** Add into the first syringe tube **0.5 mL activated sludge suspension**, **4.0 mL supernatant** and **0.5 mL** reagent **R1**.	2
5.	**Sample value:** Add into the second syringe tube **0.5 mL activated sludge suspension**, **4.0 mL sample** and **0.5 mL** reagent **R1**.	2
6.	Transfer the contents into the syringes **without air bubbles**. Seal the syringes with Luer-Lock seal plugs (female), shake and place in a test tube rack. Incubation for **2 h at room temperature in the dark**.	3 + 4
7.	Remove the seal plug and screw on the membrane filter.	5
8.	Filter the test samples and discard the filtrate. Wipe off the adherent liquid drops on the membrane filter with a disposable cloth.	5
9.	Screw on the screw plug with the suction pipe loosely to the bottle with the reagent R2. Following this screw on the two syringes with the membrane filter onto the bottle one after the other.	6
10.	**Slowly** draw off the reagent R2 via the membrane filter into the syringes up to the **marking of 4.6 mL**. Incubation for **10 min at room temperature in the dark**.	7
11.	Following this press the contents of the syringes carefully into two empty test tubes, seal the test tubes, clean them on the outside and call up **method 8902**.	8

Measurement:

For *NANOCOLOR®* photometers see manual, test 8-90.

Calculation of the relative change of the dehydrogenase activity DHA as percentage %:

$$\text{Dehydrogenase activity DHA [\%]} = [(E_S - E_R) / E_R] \times 100$$

E_R = Extinction of the reference value at 470 nm
E_S = Extinction of the sample value at 470 nm

Interpretation of the results:

- One must also calculate with a margin of error of at least ± 10 % as with the majority of biological processes. We therefore recommend the implementation of repeat determination in order to increase the dependability of the results.
- **Negative** results reveal an **inhibitive** influence of the sample, **positive** results reveal a **stimulating** influence of the sample upon the biochemical activity of sludge.
- Results of **-20 % to -80 %** *vis-à-vis* the reference value indicate a **significant level of inhibition** of the dehydrogenase activity due to the waste water sample. By means of further dilution the concentration of the waste water sample in which the biochemical activity of sludge is no longer inhibited can be determined.
- Results of **more than + 20 %** *vis-à-vis* the reference value indicate a **significant level of stimulation** of the dehydrogenase activity due to the waste water sample.
- Clear stimulation of the dehydrogenase activity of > + 20 % can occur above all in the case of nutrient rich, organically very dense waste water samples.
- The dry substance contents of the activated sludge are still not considered during the determination of the relative biochemical activity of sludge.
- Samples with a low COD content can feign inhibitions due to a lack of nutrients. In this case the supernatant of the reference value is diluted in accordance with the COD load of the sample.

Evaluation sheet
"Determination of the relative change of the dehydrogenase activity DHA" (method 8902)

Date	Sample description	E_B [E]	E_P [E]	Change of the dehydrogenase activity [%]

Method 8903: Evaluation of the degree of stabilization of sludge (Screening method)

Simple, visual process to determine the degree of stabilization of sludge (activated sludge, digested sludge) upon sewage treatment plants. The greater the progression of aerobic stabilization of sludge the more inactive the activated sludge becomes or the more progressive the putrefactiveness. This is accompanied by a decline in the conversion of TTC into red triphenylformazane.

Procedure:

Ciclo de trabajo	
1.	**Preparation of samples:** a) Determine the dry sludge mass DSM by drying at 105 °C or estimate it ± 20 % . b) The sludge which must be investigated will be diluted with sewage treatment plant water (supernatant of sedimented activated sludge) to a dry substance content level of approx. 1 g/L. **Example**: Dry sludge mass 5 g/L → target concentration: 1 g/L → 1 + 4 dilution: 1 part sludge + 4 parts sewage treatment plant water (e.g. 4 mL sludge + 16 mL sewage treatment plant water)
2.	Add **1.3 mL** reagent **R1** into an empty test tube and subsequently fills the test tube **up to the brim** and **without air bubbles** with the prepared test solution. Seal the test tube and mix it by shaking.
3.	Incubation **in the dark at room temperature**.
4.	Visually inspect the test tube after 30, 45 and 60 min for the evidence of red colouring.
5.	**Evaluation:** If **no red colouring** can be seen after **60 min** then it is generally a predominantly stabilized sludge which has reached "**technical aerobic stabilization boundary**". In the case of insufficiently stabilized sludge which is very putrefactive then a clearly recognizable red colouring can be observed after just 30 min but at the latest after 60 min.

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