

Method:	Determination of the biochemical activity of sludge (e.g. activated sludge) 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 determined photometrically.	
Ranges:	5 - 150 µg TPF 0.050 - 2.300 E	Method 8901 8902
NANOCOLOR® reagent set:	TTC / Sludge activity 150 (REF 985 890)	
Wavelength:	470 nm	
Interferences:	The triphenylformazane (TPF) which is formed is very light sensitive. Oxygen, NO ₃ ⁻ , Fe ³⁺ and NO ₂ ⁻ hinder the TTC reduction. P _i , Fe ²⁺ , SO ₄ ²⁻ , Cl ⁻ and Mn(IV) have a stimulating effect.	
Requ. accessories:	piston pipettes with tips, beaker	

Procedure:

Method 8901:

Determination of the biochemical activity of sludge (A_S)




<p>Connect a 5 ml syringe with a syringe tube by using a Luer-Lock connecting adapter female/female. Add into the syringe tube 4.5 ml activated sludge sample and 0.5 ml reagent R1.</p>
<p>The content of the syringe tube is subsequently transferred into the 5 ml syringe 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.</p>
<p>Remove the seal plug and screw on the membrane filter, colour code red. Filter the test sample and discard the filtrate.</p>
<p>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.</p>
<p>Slowly draw off the reagent R2 via the membrane filter into the syringe up to the 4.6 ml marking. Incubation for 10 min at room temperature in the dark.</p>
<p>Following this press the contents of the syringes carefully into an empty test tube. Seal the test tube, clean it on the outside.</p>

Measurement:	Call up method 8901 and perform measurement. Fill in displayed result as C_{TPF} into evaluation sheet.
Evaluation:	Determine dry sludge mass C_S at 105 °C and enter into evaluation sheet. Calculate biochemical activity A_S: A_S [µg TPF/mg] = C_{TPF} : C_S

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

Reference	Sample
Sediment the activated sludge in a suitable laboratory vessel for 30 min . Following this transfer the supernatant with a transfer pipette into a beaker.	
Connect a 5 ml syringe with a syringe tube by using a Luer-Lock connecting adapter female/female. Add into the syringe tube 0.5 ml activated sludge suspension, 4.0 ml supernatant and 0.5 ml reagent R1 .	Connect a 5 ml syringe with a syringe tube by using a Luer-Lock connecting adapter female/female. Add into the syringe tube 0.5 ml activated sludge suspension, 4.0 ml sample and 0.5 ml reagent R1 .
Transfer the contents into the syringe without air bubbles . Seal the syringes with Luer-Lock seal plugs (female). Incubation for 2 h at room temperature in the dark .	Transfer the contents into the syringe without air bubbles . Seal the syringes with Luer-Lock seal plugs (female). Incubation for 2 h at room temperature in the dark .
Remove the seal plug and screw on the membrane filter, colour code red. Filter the sample and discard the filtrate.	Remove the seal plug and screw on the membrane filter, colour code red. Filter the sample and discard the filtrate.
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.	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.
Slowly draw off the reagent R2 via the membrane filter into the syringe up to the 4.6 ml marking . Incubation for 10 min at room temperature in the dark .	Slowly draw off the reagent R2 via the membrane filter into the syringe up to the 4.6 ml marking . Incubation for 10 min at room temperature in the dark .
Following this press the contents of the syringe carefully into an empty test tube, seal the test tube, clean it on the outside.	Following this press the contents of the syringe carefully into an empty test tube, seal the test tube, clean it on the outside.

Measurement: Call up method **8902**. Extinctions are measured at 470 nm.

Place a test tube with distilled water in the cuvette slot and press key  .
Insert test tube with reference in the cuvette slot and measure the extinction E_R by pressing key  .
Insert test tube with sample in the cuvette slot and measure the extinction E_S by pressing key  .

Evaluation: Enter the extinctions **E_R** and **E_S** in the evaluation sheet. Calculate dehydrogenase activity DHA.

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

Storage: Store the reagent set dry and cool at **2–8 °C**. Please observe the expiry date!

Reference: German standard methods for the examination of water, waste water and sludge (DEV – L3 and DEV – L4)