

0–1000 mAbs (milli absorptions), 0–33.4 µmol/L (as Cytochrome C from horse heart)

**Scope and application:** For municipal wastewater and industrial wastewater.



## Test preparation

### Instrument-specific information

Table 1 shows all of the instruments that have the program for this test. The table also shows the adapter and light shield requirements for the applicable instruments that can use TNTplus vials.

To use the table, select an instrument, then read across to find the applicable information for this test.

**Table 1 Instrument-specific information for TNTplus vials**

Instrument	Adapters	Light shield
DR6000	—	—
DR3900	—	LZV849
DR1900	9609900 or 9609800 (A)	—

### Before starting

DR3900: Install the light shield in Cell Compartment #2 before this test is started.

Review the safety information and the expiration date on the package.

The recommended temperature for reagent storage is 15–25 °C (59–77 °F).

Do NOT filter sample before the digestion.

In the laboratory, pipet the sample from a stirred baffled flask to ensure turbulent mixing for representative sample quantity.

If the pipette tips become clogged with particles, a piece of the tip can be cut off to enlarge the opening.

The filtration of the digested suspension (step 11) does not have to be complete. It is sufficient, if the bottom 25 mm of the vial is filled with filtrate.

Use for filtration the recommended filters with 5–12 µm particle retention.

DR1900: Go to All Programs>LCK or TNTplus Methods>Options to select the TNTplus number for the test. Other instruments automatically select the method from the barcode on the vial.

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.

### Items to collect

Description	Quantity
TNTplus 882 vial	1
LCW906 vial	1
Reagent A	0.5 mL

## Items to collect (continued)

Description	Quantity
Permachem Reagent (Porphyrin 2) Powder Pillow	1
DRB200 reactor with 13-mm wells	1
Baffled flask	1
Magnetic stirrer with stir bar	1
Pipet, adjustable volume, 0.2–1.0 mL	1
Pipet tips, for 0.2–1.0 mL pipet	1
Pipet, adjustable volume, 1.0–5.0 mL	1
Pipet tips, for 1.0–5.0 mL pipet	1
Filter paper (5–12 µm particles kept)	1
Funnel, micro, poly	1
Test tube rack	1

Refer to [Consumable and replacement items](#) on page 4 for order information.

## Sample collection

- The biomass contains the measured substance (heme). Heme is released during the analysis. Representative sample collection is important because of the fast sedimentation of the granular ANAMMOX biomass. The largest errors occur at sample collection. Put together three or more scoop samples from the well-mixed reactor (e.g., during an aeration phase) is recommended.
- Preparation of the sample with the specified carrier materials for the biomass in the reactor (e.g., ultrasonic) is recommended.
- The sample should contain approximately 1 to 10 g/L TSS (total suspended solids). Do not put the sample through a filter before digestion.

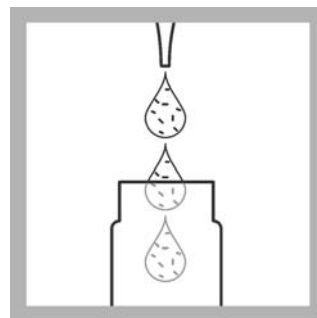
## Test procedure



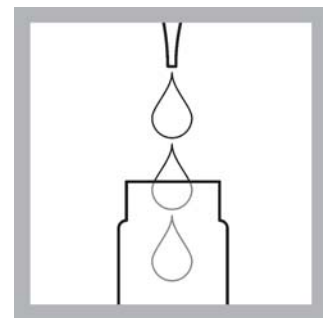
1. Set the DRB200 reactor power to on. Set the temperature to 70 °C (158 °F).



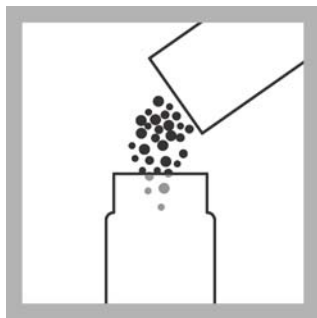
2. Stir the sample in a baffled flask with a magnetic stir plate.



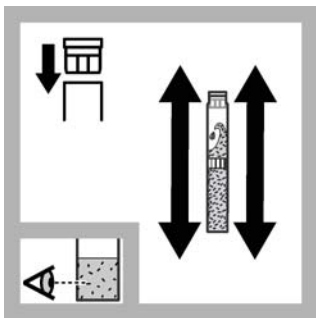
3. Use a pipet to add 5.0 mL of sample to a vial (LCW906). If a blockage occurs in the pipet tip, cut the end of the pipet tip to increase the size of the opening.



4. Use a pipet to add 0.5 mL of Reagent A to the vial.



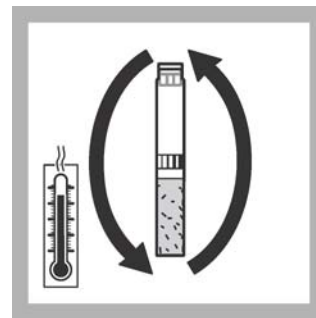
5. Add the contents of one Permachem Reagent (Porphyrin 2) Powder Pillow.



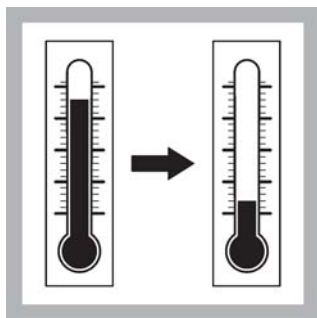
6. Put the cap on the vial. Shake to dissolve the reagent.



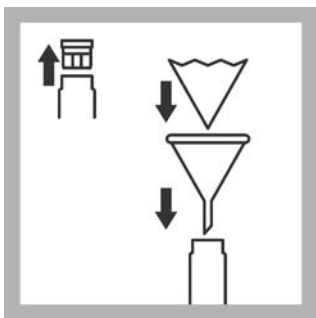
7. Clean the vial. Insert the vial in the preheated DRB200 reactor. Close the lid. Keep the vial in the reactor for 10 minutes.



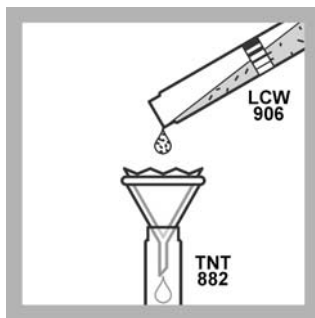
8. Hold the vial by the cap and invert gently several times while the vial is still hot.



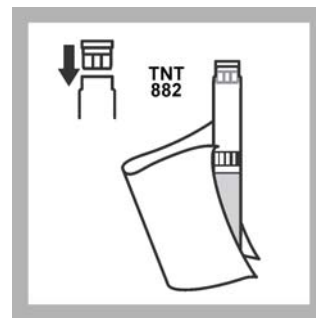
9. Put the vial in a test tube rack. Let the temperature of the vial decrease to room temperature.



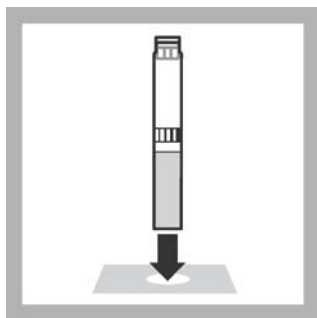
10. Remove the cap from the TNT882 vial. Put the filter paper in the funnel. Insert the funnel in the TNT882 vial.



11. Pour the digested sample into the funnel. Make sure that there is a minimum of 25 mm of filtered sample in the bottom of the vial. The sample can be turbid.



12. Put the cap on the vial. Clean the vial.



13. Insert the vial into the cell holder. DR1900 only: Select program 882. Refer to [Before starting](#) on page 1. DR1900 only: Push **READ**.

## Summary of Method

The content of heme, the protein of the ANAMMOX bacteria that contains red iron, correlates significantly with the physiological activity of ANAMMOX. Thus, the analysis of the heme content of a biomass sample is used to determine the possible ANAMMOX activity. Basic digestion removes the red pigment from the bacteria. The iron in the protein is reduced to Fe(II) and the red color is measured photometrically. The measurement wavelength is 550 nm.

## Consumable and replacement items

### Required reagents

Description	Quantity/Test	Unit	Item no.
ANAMMOX Activity, TNTplus, includes: TNTplus 882 vials (25x) and LCW906 vials (25x)	1 vial (LCW906 and TNT882)	25/pkg	TNT882KTO
Reagent A	0.5 mL	25 mL	BCF1092
Permachem Reagent (Porphyrin 2) Powder Pillow	1	100/pkg	2187569

### Required apparatus

Description	Quantity/test	Unit	Item no.
DRB200 Reactor, 115 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-01
DRB200 Reactor, 230 VAC option, 9 x 13 mm + 2 x 20 mm, 1 block	1	each	DRB200-05
Baffled flask	1	each	SM212864457
Magnetic Stirrer	1	each	2881200
Filter paper (5–12 µm particles kept)	1	100/pkg	SM10311841
Funnel, micro, poly	1	each	2584335
Test tube rack	1	each	1864100
Pipet, adjustable volume, 0.2–1.0 mL	1	each	BBP078
Pipet tips, for 0.2–1.0 mL pipet	2	100/pkg	BBP079
Pipet, adjustable volume, 1.0–5.0 mL	1	each	BBP065
Pipet tips, for 1.0–5.0 mL pipet	1	75/pkg	BBP068
Light shield, DR3900	1	each	LZV849



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