# LCK411 ANAMMOX Activity

DOC312.53.94409

## 0–1000 mAbs (milli absorptions), 0–33.4 µmol/L (as Cytochrome C) LCK411

**Scope and application:** For samples from ANAMMOX reactors and from ANAMMOX in mainstream applications.

## Test preparation

### Test storage

Storage temperature: 15–25 °C (59–77 °F)

### Items to collect

| Description | Order number |
|---|---|
| Filters (5–12 µm particle retention) | SM10311841 |
| Permachem Reagents (Porphyrin 2 Reagent) Pk/100 | 2187569 |
| Small funnel | SM106404 |
| Baffled flask | SM212864457 |
| Magnetic stirrer/Stir bar | |
| Hach Thermostat (LT200) | |

### Before starting

**Sampling:**

The substance to be analyzed (heme) is contained in the biomass and is released during the analysis. Particular attention must be paid to representative sampling, due to the rapid sedimentation of the granular ANAMMOX biomass; the largest errors may occur during this sampling step. It is recommended to combine several scoop samples from the well mixed reactor (e.g. during an aeration phase).

When using specific carrier materials for the biomass in the reactor, a suitable sample preparation (e.g. ultrasonic) is recommended. The sample should contain about 1–10 g/L TSS.

**Do NOT filter sample before the digestion.**

In the laboratory, pipet the sample from a stirred baffled flask to ensure turbulent mixing for a 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 cuvette is filled with filtrate.

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

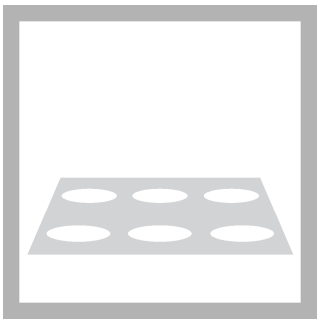
The method is applicable for DR1900, DR3900 and DR6000 only.

Review safety information and expiration date on the package.

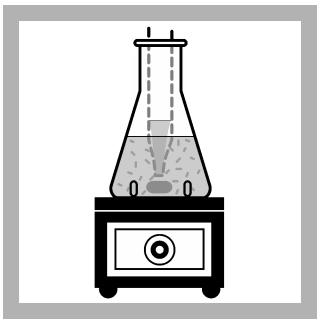
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

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.

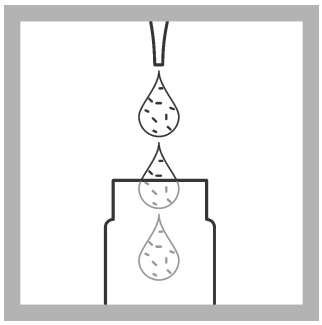
## Procedure



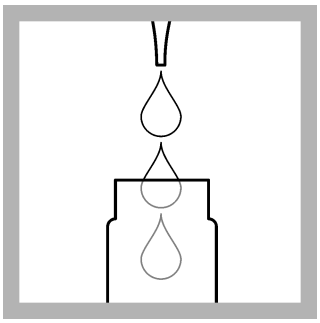
**1.** Pre-heat the thermostat **(LT200)** to 70 °C/158 °F.



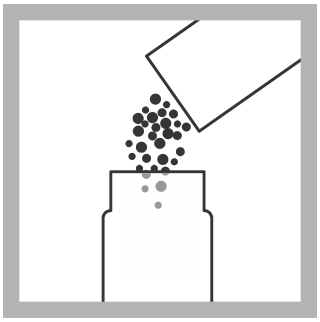
**2.** Stir the sample in a **baffled** flask while pipetting. Ensure **turbulent** mixing.



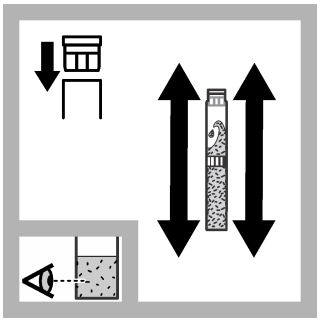
**3.** Pipet **5.0 mL sample** into a cuvette LCW906.



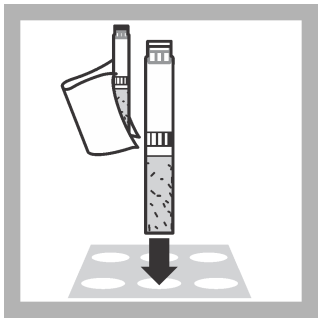
**4.** Add **0.5 mL** of **reagent A**.



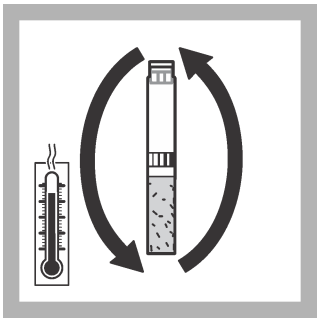
**5.** Add **1 Permachem** 2187569.



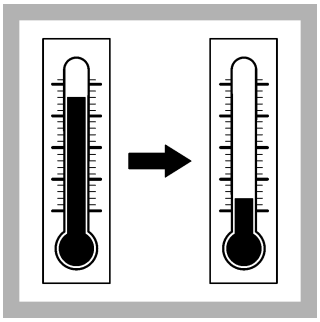
**6.** Close the cuvette and shake it until the **reagent is dissolved**.



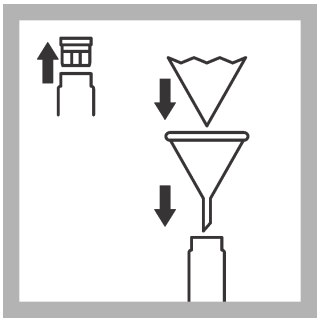
**7.** Thoroughly clean the outside of the cuvette. Place the cuvette into the thermostat:
**LT200** (pre-heated to 70 °C/158 °F): for **10 minutes** at 70 °C.



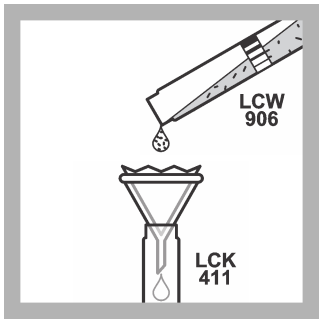
**8.** Invert the cuvette.
**Caution:** the cuvette is **still hot**.



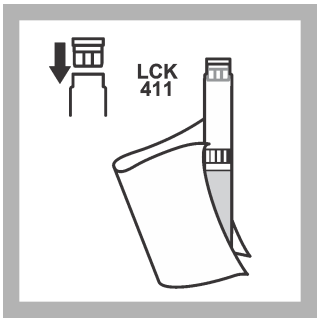
**9.** Allow to **cool** to room temperature.



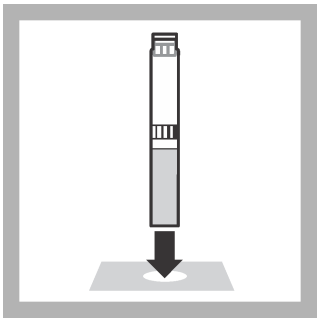
**10.** Open the cuvette. Insert a filtration paper in to the funnel and place it on a cuvette **LCK411**.




**11.** Filtrate digested sample (LCW906) into **LCK411**.
**Note:** the sample may be turbid. This does not disturb the measurement.
It is sufficient, if the bottom 25 mm of the cuvette is filled with filtrate.




**12.** Close the cuvette with a stopper.
Thoroughly clean the outside of the cuvette.



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

## Summary of method

The content of heme, the red iron containing protein of the ANAMMOX bacteria, correlates significantly with their physiological activity. The analysis of the heme content of a biomass sample, therefore allows conclusions about its potential ANAMMOX activity. The red pigment is extracted from the bacteria by alkaline digestion. The Iron in the protein is reduced to Fe(II) and the red color is measured photometrically.

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