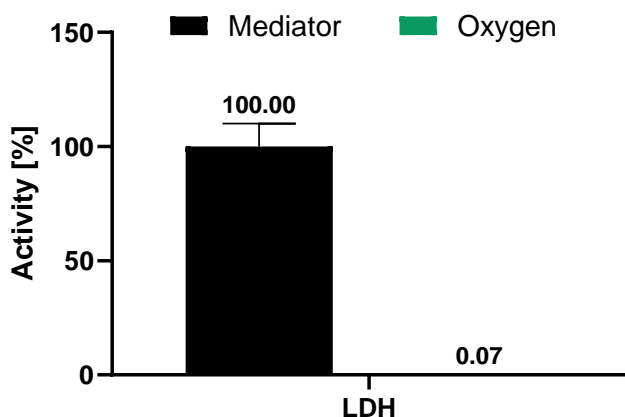




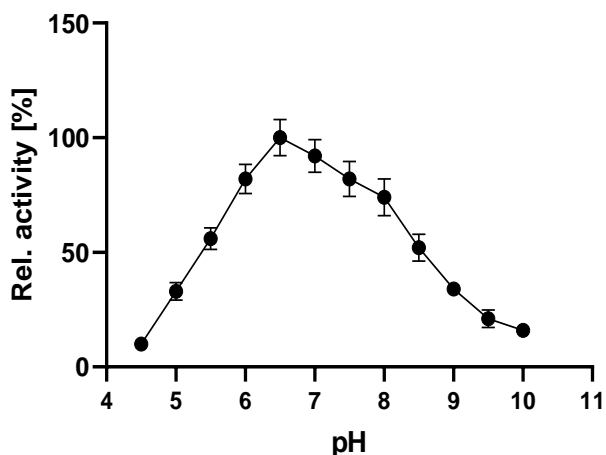
| | |
|-----------------------------------|--|
| General | LactaZyme Type: LDHLt Lactate Dehydrogenase enzyme recombinantly expressed Proprietary sequence, patent protection along EP2023/059656 |
| Structural features | FMN co-factor in the active site of the dehydrogenase domain Tetrameric. Independent on additional co-factors such as NAD(H) |
| Physical properties | Molecular weight: ~160 kDa, homotetrameric calculated Theoretical Isoelectrical point: 7.22 Extinction coefficients: at 280 nm measured in water 24870 M ⁻¹ cm ⁻¹ 0.1% (=1 g/l) = 0.599 Abs@280nm Potential Glycosylation sites unlikely |
| Formulation | Yellow powder; no additives or stabilizers Enzyme is dried from stock containing 20 mM Tris-HCl, pH 8.0 buffer Details are designated on the container and in Quality Assurance Certificate supplied with each batch. |
| Storage recommendation | Store at 4 °C. Dissolve enzyme in RO-H ₂ O. Aliquots can be prepared when dissolved and stored at -20 °C for at least 6 months Retesting is recommended every 6 months with suitable methods. NOTE: <ul style="list-style-type: none">- Single freeze / thaw cycle has shown no deactivation effect.- Minor precipitate might form when dissolved and can be removed via centrifugation. Activity is not affected. |
| Biochemical properties | Activity: ~320 U/mg Stability: T ₅₀ > 60°C, > 2 weeks at 40°C pH optimum : 6-8 |
| Electrochemical properties | Current response: > 10 µA/mm ² (Ferricyanide) Sensor shelf life: > 4 weeks at 40°C using Ferricyanide as mediator |

Electron acceptors



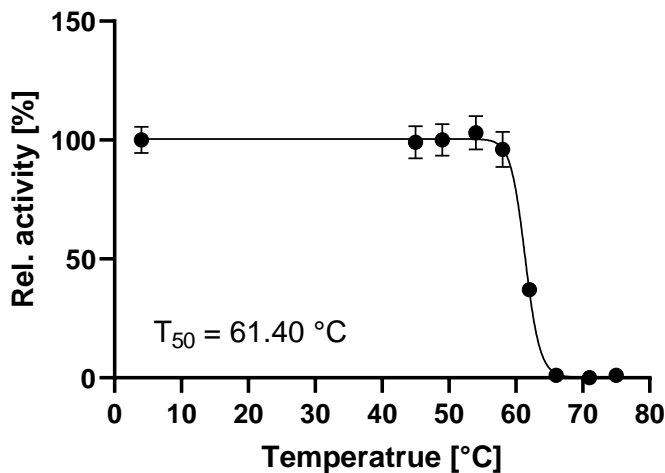
Reaction rate of enzyme assayed with the oxygen as electron acceptor in PBS buffer (red) compared to a mediator as electron acceptor (blue).

pH dependent activity



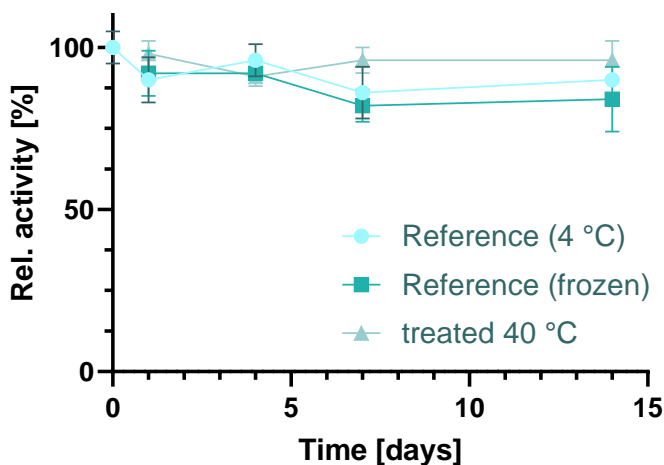
Activity of enzyme tested at various pH conditions using Britton Robinson buffer.

Thermodynamic Stability



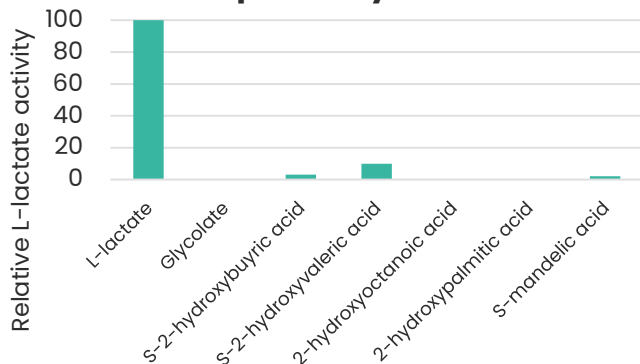
Activity of enzyme samples incubated at given temperatures for 30 min. Inset: T_{50} derived from sigmoidal fit to the data.

Shelf-life of dry enzyme



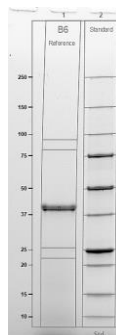
Relative activity of the enzyme after stored in dry state at typical conditions as well as elevated temperature.

Specificity



Enzyme activities assayed PBS buffer, pH 7.4, 30 °C at 10 mM of all substrates respectively.

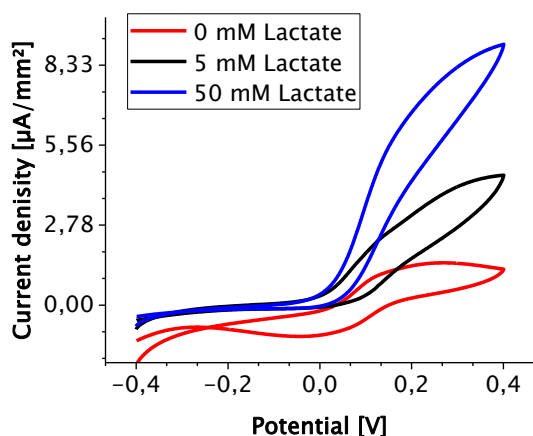
Purity



| Description | Band No. | Size (kDa) | Band % |
|-------------|----------|------------|--------|
| LDH Lt B6 | 1 | 93 | 0.1 |
| | 2 | 81 | 1.6 |
| | 3 | 42 | 95.8 |
| | 4 | 25 | 2.4 |
| | 5 | 22 | 0.5 |

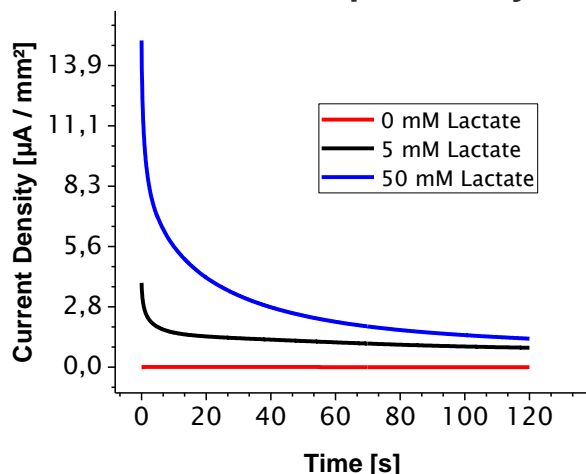
Purity and size assed by SDS PAGE and digitally processed using Image Lab.

Cyclic voltammetry



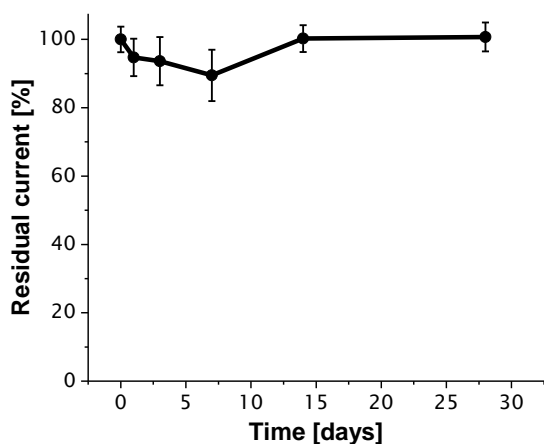
Cyclic voltammogram (10 mV s^{-1}) of the enzyme used in the biosensor reference design (WH 15)

Chronoamperometry



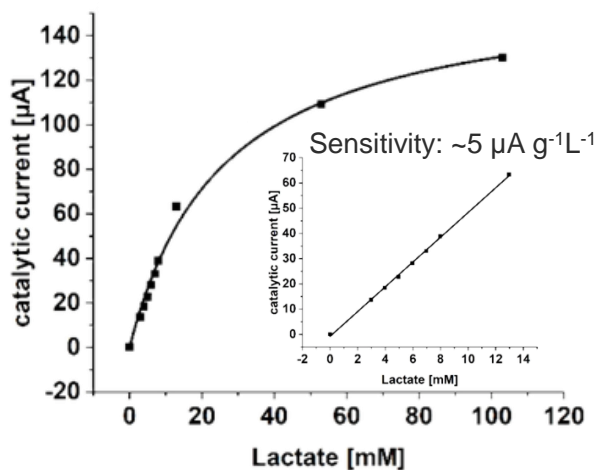
Time resolved current response to increasing lactate concentrations of the enzyme used in the biosensor reference design (WH 15)

Sensor shelf life



Current response over storage at $40 \text{ }^\circ\text{C}$ of the enzyme used in the biosensor reference design (WH 15)

Sensor calibration in Plasma



Current response to increasing lactate concentration in spiked plasma. (lyophilized citrate plasma, Sigma P9523)

Biosensor Reference Design (WH 15)

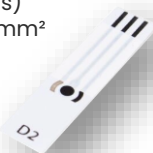
To demonstrate the bioelectrochemical performance of the enzyme, a biosensor reference design has been used. This design is intended to be simple and highly reproducible. Commercial designs may differ.

Material

Screen printed sensor: (ItalSens)
Working Electrode: Carbon 7.1 mm^2
Reference electrode: Ag|AgCl

Sensor composition

$10 \mu\text{g}$ enzyme
 $33 \mu\text{g}$ Ferricyanide (III)
0.02 % Triton
dried in silica at room temperature 2 h



Measurement Setup

Add $100 \mu\text{l}$ lactate dissolved in buffer (50 mM Phosphate, 140 mM NaCl, pH 7.4)
Apply $+250 \text{ mV}$ to horizontally mounted electrode.
Record current response over time and evaluate.