

Claims

- 5 1. A method of measuring the pH of a solution, wherein the method comprises the steps of:
- providing a solution comprising a quinone, a quinone derivative, and/or a pH indicator, wherein the quinone, quinone derivative, and/or pH indicator is dissolved in the solution;
 - applying the solution to a three-electrode electrochemical cell;
 - 10 - measuring an electrochemical response of the electrochemical cell; and
 - quantifying the pH of the solution as a function of the electrochemical response of the electrochemical cell, ~~wherein the response correlates with the electrochemical state of the quinone, quinone derivative and/or pH indicator.~~
- 15 2. The method according to Claim 1, wherein the quinone or quinone derivative is selected from the list consisting of 1,2-benzoquinone, 1,4-benzoquinone, 1,4-naphthoquinone, 9,10-anthraquinone, and derivatives and combinations thereof, and
- 20 ~~3. The method according to claim 1~~ wherein the pH indicator is selected from the list consisting of malachite green oxalate, brilliant green, eosin yellowish, erythrosine B, methyl green, methyl violet, picric acid, cresol red, crystal violet, m-Cresol purple, thymol blue, p-Xylenol blue, Eosin (bluish), quinaldine red, 2,4-dinitro phenol, 4-(dimethylamino) azobenzol, bromochlorophenol blue, bromophenol blue, congo red, methyl orange, bromocresol green, 2,5-dinitrophenol, alizarin sulphonic acid, methyl red, chlorophenol red, litmus, bromocresol purple, bromophenol red, 4-nitrophenol, bromoxyleneol blue, bromothymol blue, phenol red, 3-nitrophenol, neutral red, creosol red, 1-naphtholphthalein, m-cresol purple, thymol blue, p-xylenol blue, phenolphthalein, thymolphthalein, alkali blue, alizarin yellow GG, indigo carmine, epsilon blue, titan yellow, and combinations thereof.
- 25 3. The method according to any one of the preceding claims, wherein the electrochemical response of the electrochemical cell that is measured is the potential of the electrochemical cell, the current of the electrochemical cell, the impedance of the electrochemical cell, or a combination of these.
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5.4. The method according to any one of the preceding claims, wherein the electrochemical response of the electrochemical cell that is measured comprises a combination of the potential of the electrochemical cell, the current of the electrochemical cell, the impedance of the electrochemical cell.

6.5. The method according to any one of the preceding claims, wherein the potential of the electrochemical cell is measured via cyclic squarewave voltammetry, squarewave voltammetry, linear sweep voltammetry, cyclic voltammetry or open circuit potentiometry.

7.6. The method according to any one of the preceding claims, wherein the concentration of the quinone, a quinone derivative, and/or a pH indicator within the solution is from about 1 μ M to about 1000 μ M, such as from about 1 μ M to about 500 μ M, for example from about 5 μ M to about 250 μ M, in particular from about 50 μ M to about 200 μ M.

~~8. The method according to any one of the preceding claims, wherein the step of quantifying the pH of the solution as a function of the electrochemical response of the electrochemical cell is performed via application of a regression algorithm.~~

9.7. The method according to any preceding claim, wherein the solution comprises a buffer, and wherein the solution further comprises:

~~10. The method according to any preceding claim, wherein the solution further comprises a sample, preferably wherein the sample is selected from the list consisting of a nasal sample, a throat sample, an anal sample, a vaginal sample, an ear draining sample, a skin surface swab sample, a urine sample, a whole blood sample, a serum sample, a plasma sample and a lymph drainage sample; and.~~

~~11. The method according to any preceding claim, wherein the solution further comprises primers and nucleic acid amplification reagents, preferably wherein the nucleic acid amplification reagents are LAMP reagents.~~

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~~12. The method according to Claim 11, wherein the nucleic acid amplification reagents comprise a signaling substance, or are capable of releasing a signaling substance, or comprise both a signaling substance and a substance capable of release a signaling substance.~~

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~~13. The method according to claim 12, wherein the signaling substance is H⁺.~~

~~14.8.~~ The method according to any one of claims 11 to 13~~claim 7~~, wherein the method comprises a step of performing a nucleic acid amplification step, preferably a plurality of nucleic acid amplification steps.

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~~15.9.~~ The method according to any one of claims 11 to 14~~7 to 8~~, wherein the nucleic acid amplification step(s) is/are a loop-mediated isothermal amplification (LAMP) step, and

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~~16. The method according to any one of claims 11 to 15, wherein the method is for determining the presence of a target polynucleotide in the solution, and wherein the nucleotide amplification is performed at a constant temperature in the range of from 59 to 75°C, such as from 62 to 73°C, such as from 64 to 70°C, such as from 66 to 68°C.~~

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~~17. The method according to any one of claims 11 to 16, wherein the step of measuring the electrochemical response of the electrochemical cell comprises measuring a change in the current and/or potential of the electrochemical cell due to a concentration change of the signaling substance.~~

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~~18.10.~~ The method according to any one of the preceding claims, wherein said method quantifies a change in pH of the solution.

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~~19. The method according to any one of claims 11 to 18, wherein the LAMP reagents comprise a reverse transcriptase.~~

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5 ~~20. The method according to any one of claims 11 to 19, wherein the primers comprise a forward inner primer, a backward inner primer, a forward outer primer and a backward outer primer.~~

10 ~~21. The method according to claim 20, optionally wherein the LAMP amplification is performed with an additional pair of loop primers, such as forward loop primer and/or backward loop primer.~~

15 ~~22.11. The method according to any of the preceding claims, wherein the three-electrode electrochemical cell comprises a working electrode, a reference electrode and a counter electrode.~~

20 ~~23. The method according to any one of the preceding claims, wherein the working electrode comprises an ion selective membrane, located such that it excludes selected ions of the solution from contacting the working electrode.~~

24. ~~The method according to any one of the preceding claims, wherein the electrodes comprise or consist of gold, silver, carbon, platinum, ruthenium dioxide, or a combination thereof, and~~

25 ~~25. The method according to any one of the preceding claims, wherein the material of the electrodes are identical or different.~~

30 ~~26.12. The method according to any one of the preceding claims, wherein the electrodes of the three-electrode electrochemical cell are film electrodes, such as screen-printed electrodes or cell are wire electrodes.~~

27. ~~The method according to any one of the preceding claims, wherein the electrodes of the three-electrode electrochemical cell are wire electrodes.~~

35 ~~28. The method according to any one of the preceding claims, wherein the three-electrode electrochemical cell comprises a substrate onto which the electrodes~~

are located, and wherein the material of said substrate is a chemically inert material, such as alumina, ceramic, glass, or an inert plastic.

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29. The method according to any one of the preceding claims, wherein the three-electrode electrochemical cell comprises a microfluidic inlet, in fluidic connection with the electrochemical cell, for receiving the solution.

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30. The method according to any one of the preceding claims, wherein, following application of the solution, a vapour barrier is added for preventing evaporation of the solution.

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31. The method according to claim 30, wherein the vapor barrier is formed by fluidly sealing the inlet of the three-electrode electrochemical cell, or the microwell, with a physical lid, a heated lid, a mineral oil, and/or a paraffin wax.

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32. The method according to any one of the preceding claims, wherein a hydrophilic area connects the microfluidic inlet and the three-electrode electrochemical cell, such as for capillary filling of the three-electrode electrochemical cell.

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~~33.13.~~ The method according to any one of the preceding claims, wherein the measurements are performed by using a potentiostat or similar circuit, and.

34. The method according to any of the preceding claims, wherein the measurements are performed for a period of 1 minute to 90 minutes.

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35. The method according to any of the preceding claims, wherein the nucleotide amplification is performed at a constant temperature in the range of from 59 to 75°C, such as from 62 to 73°C, such as from 64 to 70°C, such as from 66 to 68°C.

36.14. Use of a three-electrode electrochemical cell for measuring the pH of a solution, said use comprising:

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- providing a solution comprising a quinone, a quinone derivative, a pH indicator, or combinations thereof, wherein the quinone, quinone derivative, and/or pH indicator is dissolved in the solution;
 - applying the solution to the three-electrode electrochemical cell;
 - measuring an electrochemical response of the electrochemical cell; and
 - quantifying the pH of the solution as a function of the electrochemical response of the electrochemical cell, ~~wherein the response correlates with the electrochemical state of the quinone, quinone derivative and/or pH indicator.~~
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~~37.15.~~ Use of a three-electrode electrochemical cell for monitoring a nucleic acid amplification reaction, such as loop-mediated isothermal amplification (LAMP), said use comprising:

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- providing a solution comprising a quinone, a quinone derivative, a pH indicator, or combinations thereof, wherein the quinone, quinone derivative, and/or pH indicator is dissolved in the solution;
 - providing a LAMP mixture comprising: a sample comprising a target polynucleotide, at least two, such as at least four primers each flanking the target polynucleotide and LAMP reagents;
 - applying the solution and the LAMP mixture to the three-electrode electrochemical cell;
 - measuring an electrochemical response of the electrochemical cell; and
 - quantifying the pH of the solution as a function of the electrochemical response of the electrochemical cell, ~~wherein the response correlates with the electrochemical state of the quinone, quinone derivative and/or pH indicator.~~
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~~38. The use according to claim 37, wherein the amplification reaction is loop-mediated isothermal amplification (LAMP).~~

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~~39. Use of a system comprising:~~

- ~~a. a potentiostat; and~~
- ~~b. a measuring kit, said kit comprising:~~

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i. ~~a first receptacle comprising a three-electrode electrochemical cell, and a second receptacle comprising a quinone, a quinone derivative, and/or a pH indicator, or~~

ii. ~~a first receptacle comprising a three-electrode electrochemical cell and a quinone, a quinone derivative, and/or a pH indicator,~~

~~wherein the quinone, quinone derivative, and/or pH indicator are dissolved in an aqueous solution in the first or in the second receptacle,~~

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~~for measuring the pH of a solution.~~

40.16. A system for measuring the pH of a solution, the system comprising:

a. a potentiostat;

b. a measuring kit, said kit comprising:

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i. ~~a first receptacle comprising a three-electrode electrochemical cell, and a second receptacle comprising a quinone, a quinone derivative, and/or a pH indicator, or~~

ii. ~~a first receptacle comprising a three-electrode electrochemical cell and a quinone, a quinone derivative, and/or a pH indicator,~~

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~~wherein the quinone, quinone derivative, and/or pH indicator are dissolved in an aqueous solution in the first or in the second receptacle, and~~

~~wherein the first receptacle accommodates a nucleic acid amplification reaction system, such as at least four primers configured to flank a target polynucleotide sequence and LAMP reagents; and~~

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c. a heating unit, configured for heating the first/or second receptacle, such as ~~for heating the first receptacle to a temperature within the range of 59°C to 75°C.~~

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17. The system according to claim 16, wherein the quinone, quinone derivative, and/or pH indicator are dissolved in an aqueous solution in the first or in the second receptacle.

~~41. The system according to claim 40, wherein the potentiostat is configured to measure an electrochemical response of the electrochemical cell.~~

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~~42.18.~~ The system according to any one of claims 40 and 41 the preceding claims,
wherein the potentiostat is configured to measure an electrochemical response,
wherein the electrochemical response is representative of the oxidation state of
the quinone, quinone derivative, and/or pH indicator in the first receptacle.

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19. The system according to any one of the preceding claims, wherein the first
receptacle accommodates a nucleic acid amplification reaction system, such as
at least four primers configured to flank a target polynucleotide sequence and
LAMP reagents.

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~~43.20.~~ The system according to any one of claims 40 to 42 the preceding claims,
wherein the first receptacle comprises a microfluidic inlet for receiving a solution
and/or a sample.

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~~44. The system according to claim 43, wherein the microfluidic inlet is connected to
the electrochemical cell by a hydrophilic zone, such as a porous/fibrous structure
or a hydrophilic channel, configured to transport the sample by capillary action.~~

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~~45. The system according to any one of claims 40 to 44, wherein the first receptacle
and/or the second receptacle is an Eppendorf tube, a microwell, or a culture flask.~~

~~46. The system according to any one of claims 40 to 45, wherein the electrodes are
as defined in any one of claims 22 to 29.~~