(19) Europäisches Patentamt
European Patent Office
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(11) **EP 4 292 714 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
20.12.2023 Bulletin 2023/51

(21) Application number: **22179190.8**

(22) Date of filing: **15.06.2022**

(51) International Patent Classification (IPC):
B01L 7/00 *(2006.01)* ***B01L 9/06*** *(2006.01)*
C12Q 1/68 *(2018.01)*

(52) Cooperative Patent Classification (CPC):
B01L 7/52; B01L 9/06; B01L 2300/0654;
B01L 2300/1822

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
Designated Validation States:
KH MA MD TN

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(54) APPARATUS FOR RAPID PCR ANALYSIS

(57) The present invention relates to a PCR apparatus for nucleic acid amplification comprising four main modules - a power supply module, a temperature module, fluorescence excitation-detection module, control module wherein the temperature module contains thermocyclic plate made of graphite allowing to achieve higher heating/cooling speeds for faster polymerase chain reactions and allowing to form reaction products more quickly. Graphite is a material with high thermal conductivity and low specific heat capacity making it suitable for ultra-fast temperature changes.

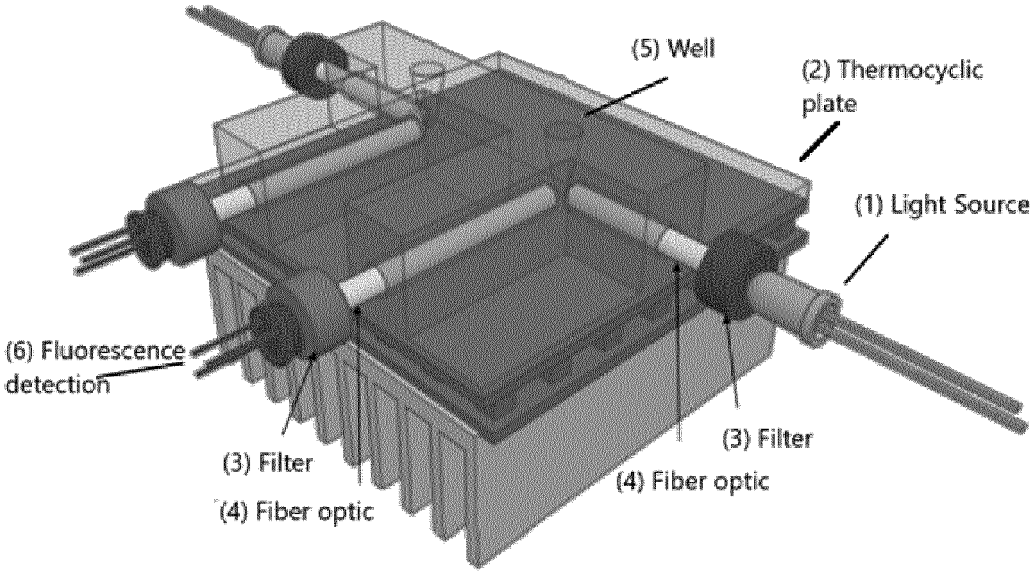

Fig. 1

EP 4 292 714 A1

Description

Technical field

[0001] The present invention relates to a PCR apparatus for nucleic acid amplification.

Background art

[0002] The polymerase chain reaction (PCR) is widely used by research professionals around the world to amplify small strands of DNA. Typically, PCR is performed using automated thermal cyclers that alternately heat and cool numerous small tubes containing the PCR reaction mixture. Since the invention of the PCR, numerous designs for thermocycling devices have been developed in an effort to increase the speed of nucleic acid amplification. The need for rapid methods and devices for examining genetic material became particularly apparent during the pandemic. One way to increase the speed of PCR reaction is to use thermocyclic plate (2) made of material that allows the temperature to increase and drop as fast as possible. In the analysis of various temperature characteristics of materials suitable for ultra-fast temperature change, a graphite was selected - a material with high thermal conductivity and low specific heat capacity. When comparing graphite with aluminum, it was found that graphite thermocyclic plate (2) of the same shape (equal volume) requires about 40% less energy compared to aluminum thermocyclic plate (2). Graphite allows for higher heating/cooling rates compared to commonly used aluminum. The closest prior art document CN203921614U uses a thermocyclic plate (2) comprising thin graphite elements between layers of other materials. The apparatus described herein uses a thermocyclic plate (2) made entirely of graphite allowing faster temperature changes.

Brief description of drawings

[0003] Fig. 1 3D representation of the apparatus for nucleic acid amplification.

Detailed description

[0004] The device consists of four main modules:

1. Power supply module.
2. Temperature module.
3. Fluorescence excitation-detection module.
4. Control module.

[0005] The power supply module consists of a high-current battery and an external power supply / charging unit. The PCR device can operate on both internal battery power and an external power supply. The internal battery is equipped with a protection module that controls the charging / discharging currents, balances the charging of the cells, protects against excessive discharge. The external power supply / charging unit can generate up to 22A at 16.8 V. The external operation of the power supply is organized in such a way that the batteries are also charged when the device is operated from an external power supply. The control module constantly monitors the battery charge level and displays it on the screen. If the charge level is insufficient for one procedure, the control module will not start the procedure until the unit is connected to an external power supply or charged enough to perform at least 1 complete procedure.

[0006] The temperature module consists of a thermocyclic plate (2), a heating / cooling element and an active heat dissipation system. In the analysis of various temperature characteristics of materials suitable for ultra-fast temperature change, a material with high thermal conductivity and low specific heat capacity - graphite - was selected. When comparing graphite with aluminum, it was found that graphite thermocyclic plate (2) of the same shape (equal volume) requires about 40% less energy compared to aluminum thermocyclic plate (2). Graphite allows for higher heating / cooling rates compared to commonly used aluminum. The plate was provided with 2 wells (5) for 0.2 ml tubes 2 channels for fluorescence excitation and 2 channels for fluorescence collection. To further reduce thermal inertia, the thermocyclic plate (2) design was optimised to have high surface area to mass ratio. Depending on the mass of the thermocyclic plate (2) and the required rate of temperature change for heating / cooling, a 430W Peltier element was selected. To achieve the highest heat transfer coefficient between the thermocyclic plate (2), the Peltier element, the passive cooling element and a metallized thermal paste was used. The active heat dissipation system consists of an aluminum radiator and an active fan that blows heat away from the radiator. The active cooling element is selected to dissipate 450W of the heat generated by cooling the thermocyclic plate (2).

[0007] The most efficient energy transfer conditions of the Peltier element were maintained to ensure the maximum rate of temperature change. The energy transfer of the damper element is highest when the temperature difference between the hot and cold sides is smallest. As a result, the radiator temperature was actively controlled by regulating the fan speed.

[0008] Evaporation of the solution occurs when the sample is heated. Due to the extremely small volume of the sample, all of the solution evaporates and amplification does not occur, although the liquid condenses and drains to the bottom of the tube as it cools. For this purpose, a higher temperature must be maintained at the top of the tube to prevent evaporation. Amplification experiments showed that the temperature difference between the enclosure and the maximum thermocyclic plate (2) should be in the range of 5-10 °C for optimal amplification efficiency.

[0009] The fluorescence excitation-detection module consists of light sources (1), filters (3) and detectors (6).

The components of a fluorescence excitation detection system are closely related. Excitation should be selected in the spectral range where dye absorption is highest and recording in the spectral range where dye fluorescence is most intense. However, the absorption and fluorescence bands overlap and these areas of maximum intensities are adjacent to each other at a distance of about 20 to 30 nm, so that with conventional color filters, the excitation light enters the detector (6), thus reducing the overall sensitivity of the system. Depending on the dyes to be used, a combination of LED and excitation / detection filters (3) was chosen to ensure no overlap in the excitation and detection areas and a high sensitivity for fluorescence detection. The last important component in the fluorescence excitation detection module is the detector (6). During the test, the volume of the test solution is very small (-10 ul) and the fluorescence intensity is very low, so the detector (6) must be extremely sensitive. At the same time, the detector (6) must be fast enough to record fluorescence at a stable temperature point. A fast, high-sensitivity, PIN-type photodiode has been selected for this purpose. The photovoltaic diode operating mode is selected to reduce the noise generated by the dark current. To increase the recording efficiency of the photodiode, it was mounted behind a thermocyclic plate (2) and the fluorescence from the tube to the photodiode was "transported" by a fiber optic (4) system that additionally acts as a spatial filter to reduce the effects of reflections (Add a detailed light removal filtration scheme). The photodiodes are additionally actively cooled. For the registration of extremely low currents, the photodiodes were equipped with FET-type two-stage operational amplifiers, additionally shielding them from external electromagnetic fields using the Faraday cage principle. In the first stage, the current of the photodiode is converted into a voltage, and in the second stage, this voltage is increased.

[0010] The control module consists of 5 parts - heating / cooling element controller, passive coolant temperature controller, temperature feedback controller, fluorescence controller and process controller. The requirements for the heating / cooling element controller are - fast and accurate control of high current in both directions of current flow with minimal heat loss. A multitransistor current control model was chosen for this. The passive coolant temperature controller controls the active passive coolant temperature controller, thus ensuring the most efficient energy transfer of the Peltier element. The purpose of the temperature feedback controller is to periodically record the thermocyclic plate (2) temperature at high speed and high accuracy and transmit the information to the process controller. The process controller has an Integrated ADC converter that digitizes the incoming signal and transmits it for further processing. The fluorescence controller consists of two modules - the excitation source controller and the detection module controller. The excitation source controller must be stabilized to ensure uniform excitation intensity as operating conditions change. The controller of the detection module must ensure the amplification of the weak fluorescence signal and efficient noise filtering. One of the most complex components of a device is the process controller. It must be able to receive information from different input devices, process digital and analog signals, control different types of controllers, and present the processed information to output devices. A program and a set of algorithms were written for parameter registration, analysis and process control. Fuzzy logic algorithm was used to control the temperature of the thermocyclic plate (2). The algorithm compares the measured actual thermocyclic plate (2) temperature with the set one, which depends on the selected test type. The difference between the measured and set temperature is multiplied by a fixed constant and converted to time. This time is used to generate a modulation control signal (PWM) for the Peltier module controller.

[0011] The temperature in the thermocycler is controlled by recording the temperature of the thermocyclic plate (2). Meanwhile, the contact of the tube with the thermocyclic plate (2) and a certain coefficient of temperature transfer to the solution results in temperature differences between the thermocyclic plate (2) and the solution. The temperature in the tube is - 2-5 degrees different from the temperature of the thermocyclic plate (2) in the apparatus. After about 2-3 seconds, the temperature in the tube reaches the set value. Taking into account these temperature differences, the temperature control algorithm was adjusted - the temperature of the plate is raised to such values (experimental and modeling data) that the solution in the cuvette reaches the temperature specified in the procedure.

[0012] Principle of operation. The instrument is turned on and the test type is selected (procedure type with pre-recorded specific number of cycles, temperature settings, fluorescence recording settings). One type of study is manual, i.e. it is possible to fully select all parameters according to the user's needs. The battery charge is estimated and in the event of an insufficient charge level, the selected procedure is notified to the user, the procedure is not allowed to run on battery power only, and the procedure is suggested to be performed when connected to an external power source. If the initial conditions are met, the procedure is initiated. The instrument asks you to add a sample. After inserting the sample, the instrument checks that the tube is inserted and after closing the lid, asks you to confirm with the button on the display that we are starting the amplification procedure. The appliance rolls up or the lid is properly (completely) closed and the hood starts to heat up. The hood is heated to maximum capacity. When the enclosure temperature reaches 80% of the default temperature, the heating of the main thermoblock is switched on. When both the hood and the main thermoblock reach the set temperatures, a cyclic process is started, the parameters of which are pre-programmed. The instrument control is programmed so that the time and temperature parameters of the cycle

can be changed within the ranges specified in the instrument characteristics, and fluorescence recording is possible at any stage of the cycle. This provides opportunities to perform unique experiments in the study of the mechanisms of dye intercalation into a DNA molecule. The display always shows the number of cycles and, depending on the settings, a curve of the fluorescence intensity and the fluorescence value of the current cycle. The data is written to the memory chip in real time. After the procedure (all cycles have been completed), the temperature maintenance devices are switched off. The screen informs you of the end of the procedure, analyzes the data, and displays the result - the answer to the clinical question / task. If necessary, the next procedure can be started immediately by selecting the start new procedure button on the device screen. When this option is selected, you are again asked to select the type of procedure you want to perform and the sequence of steps described above is initiated.

Claims

1. An apparatus for nucleic acids amplification, comprising:

   a power supply module comprised of a battery and/or an external power supply and/or charging unit for supplying power to the apparatus;
   a fluorescence excitation-detection module comprised of one or more light source (1), filter (3) and detector (6) for measuring an amount of product formed;
   a control module for monitoring charge level; and
   a temperature module comprised of a thermocyclic plate (2) and a heating/cooling element;
   characterised in that the thermocyclic plate (2) is made of graphite allowing to achieve higher heating/cooling speeds for faster polymerase chain reactions and allowing to form reaction products more quickly.

2. The apparatus of claim 1, wherein graphite thermocyclic plate (2) further comprises one or more wells (5) configured to hold a sample wherein the wells (5) are configured to hold one or more 0.2 ml tubes.

3. The apparatus of claim 1, wherein graphite thermocyclic plate (2) comprises one or more channels for fluorescence excitation and detection.

4. The apparatus of claim 1, wherein the heating/cooling element is Peltier element.

5. The apparatus of claim 1 and 5, wherein the Peltier element is 430W.

6. The apparatus of claim 1, wherein a metallized thermal paste is used between the thermocyclic plate (2) and a passive cooling element.

7. The apparatus of claim 1, wherein the power is supplied either by an internal battery or external power supply.

8. The apparatus of claim 1, wherein the control module continuously monitors and displays charge level.

9. The apparatus of claim 8, wherein the control module does not allow to start PCR reaction until the apparatus has a sufficient level of charge for at least a single PCR reaction or is connected to an external power supply.

10. The apparatus according to claim 1, wherein the thermocyclic plate (2) temperature is controlled using the fuzzy logic algorithm.

11. The apparatus according to claim 1, for use in dye intercalation to DNA molecules.

12. A method of using the apparatus of claim 1 comprising:

    collecting a fluid sample;
    disposing the fluid sample within one or more wells (5) of the thermocyclic plate (2);
    amplifying DNA using repeated cycles of heating and cooling.

13. The method of claim 13 for use to detect a gene variant of the CYP4F2 enzyme (rs3093135).

14. A thermocyclic plate (2) made of graphite according to any preceding claims that allows to increase and decrease temperature more quickly allowing for polymerase chain reactions to occur faster.

15. A fiber optics (4) used for fluorescence excitation and detection light paths to reduce thermal effects on light source (1) and detectors (6) and to realize light spatial filtration to increase spectral filtration effectiveness and consequently increase signal to noise ratio.

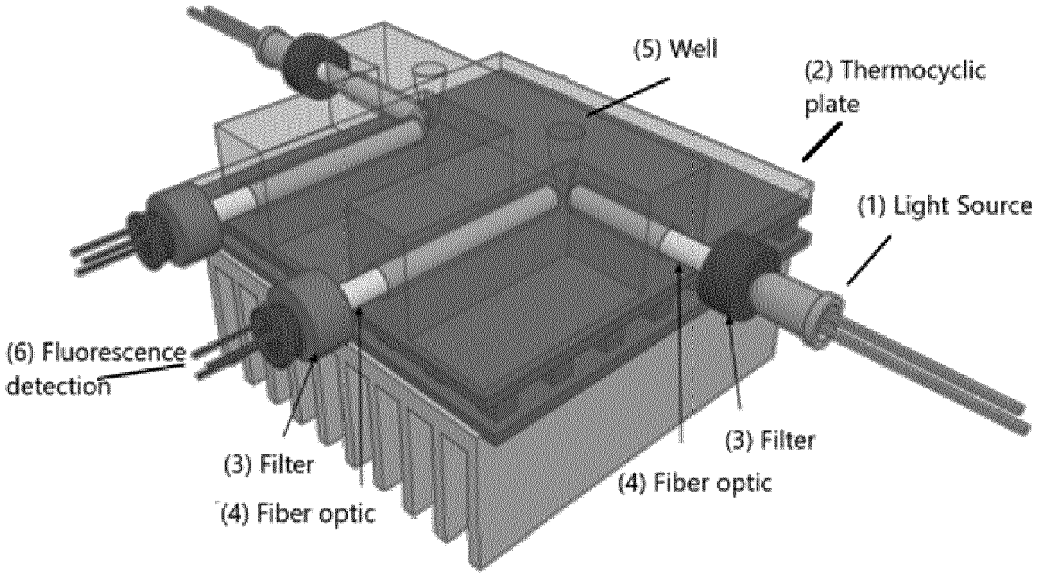
(5) Well
(2) Thermocyclic plate
(1) Light Source
(6) Fluorescence detection
(3) Filter
(4) Fiber optic
(3) Filter
(4) Fiber optic

Fig. 1

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EUROPEAN SEARCH REPORT

Application Number
EP 22 17 9190

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	23 November 2022	Campbell, Paul

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