

OBJECTS AND SUMMARY OF THE INVENTION

[0016]

In view of the above, an object of the present invention is to provide a kit for detecting multiple target nucleic acids capable of simultaneously amplifying and detecting
5 multiple genes by means of one reaction vessel containing one kind of reaction solution and one label.

[0017]

A first aspect of the present invention provides a kit for together detecting multiple target nucleic acids differing from each other, comprising: solution, the multiple target
10 nucleic acids including a first target nucleic acid and a second target nucleic acid; the solution being capable of containing the first target nucleic acid and the second target nucleic acid therein, at a denaturation temperature T0, first double-stranded hydrogen bond of the first target nucleic acid being cut off to be dissociate into first two single strands, second double-stranded hydrogen bond of the second target nucleic acid being
15 cut off to be dissociate into second two single strands, respectively, the solution further containing therein: a first target's primer at an annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated; a second target's primer at the annealing temperature T1 specifically bonding with either of the second two single strands into which the second
20 target nucleic acid has been dissociated; a first target's probe at the annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated, the first target's probe including a first labeling substance changing first fluorescent signals thereof when the first target's probe specifically bonds with either of the first two single strands; DNA polymerase;
25 deoxyribonucleoside triphosphate at an elongation temperature T2 bonding by action of the DNA polymerase with both of the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into which the

second target nucleic acid has been dissociated; and a second target's probe at the annealing temperature T1 bonding with neither the first two single strands into which the first target nucleic acid has been dissociated nor the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe at a
5 second target detection temperature T3 bonding with the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe including a second labeling substance changing second fluorescent signals thereof when the second target's probe specifically bonds with either of the second two single strands, characterized in that:

10 T0 is defined as the denaturation temperature; T1 is defined as the annealing temperature; T2 is defined as the elongation temperature; and T3 is defined as the second target detection temperature, T0 through T3 being set up such that a condition that: $T0 > T2 \geq T1 > T3$ is satisfied; and

the first labeling substance is equal to the second labeling substance so that the
15 first fluorescent signals are also equal to the second fluorescent signals.

A second aspect of the present invention provides a detection method, comprising: using the kit for together detecting multiple target nucleic acids differing from each other as defined in the first aspect; a dissociating step of increasing temperature of the solution to the denaturation temperature T0 so as to dissociate the first target nucleic acid into
20 the first two single strands, and to dissociate the second target nucleic acid into the second two single strands respectively; a first target nucleic acid detecting step of decreasing the temperature of the solution to the annealing temperature T1 so as to detect whether or not the first target nucleic acid is contained in the solution based on the first fluorescent signals shown by the first target's probe; an amplification step of

setting up the temperature of the solution to the elongation temperature T2 so as to amplify the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into which the second target nucleic acid has been dissociated, respectively; and a second target nucleic acid detecting step of
5 decreasing the temperature of the solution to the second target detection temperature T3 so as to detect whether or not the second target nucleic acid is contained in the solution based on the second fluorescent signals shown by the second target's probe.

[0018]

It is preferable that each of the first labeling substance and the second labeling
10 substance is selected from a group consisting of: a QProbe (registered trademark) probe; an Eprobe (registered trademark) probe; and a TaqMan (registered trademark) probe.

[0019]

The fluorescent signals may be shown by quenching light when the annealing occurs. Alternatively, the fluorescent signals may be shown by emitting light when the
15 annealing occurs.

[0020]

It is preferable that the first target nucleic acid is at least one of a *Mycoplasma pneumoniae* P1 gene and a *Chlamydia trachomatis* endogeneous plasmid gene, and the second target nucleic acid is at least one of internal control composition and a *Nisseria gonorrhoeae* CMT gene, respectively.
20

Effect of Invention

[0021]

target's F-primer 23 specifically bonds with a complementary sequence of the first single strand 21, and a second target's R-primer 24 specifically bonds with another complementary sequence of the second single strand 22, respectively.

[0029]

5 At the annealing temperature T1 shown in Fig. 37 (b), the first target's probe 15 labeled by means of the first labeling substance 16 specifically bonds with a specific part of the first single strand 11 derived from the first target nucleic acid 10, thereby the first labeling substance 16 outputs first fluorescence signals.

On the other hand, the annealing temperature T1 is higher than the second target
10 detection temperature T3. For this reason, the second target's probe 25 labeled by means of the second labeling substance 26 does not bond with the first single strand 21 derived from the second target nucleic acid 20, thereby the second labeling substance 26 outputs no fluorescence signal at this time.

[0030]

15 As shown in Fig. 37 (c), when the temperature is increased from the annealing temperature T1 to the elongation temperature T2, amplification reaction progresses advances as follows. This is because the solution 2 contains an amount sufficient for repeating PCR cycles of DNA polymerase 30 and deoxyribonucleoside triphosphate 31.

[0031]

20 Regarding the first target nucleic acid 10, from the first target's F-primer 13 bonding with the first single strand 11, and from the first target's R-primer 14 bonding with the second single strand 12, the deoxyribonucleoside triphosphate 31 bonds therewith to elongate, respectively.

[0032]

25 Regarding the second target nucleic acid 20, from the second target's F-primer 23 bonding with the first single strand 21, and from the second target's R-primer 24 bonding with the second single strand 22, the deoxyribonucleoside triphosphate 31 bonds

- 13: First target's F-primer
- 14: First target's R-primer
- 15: First target's probe
- 16: First labeling substance
- 5 20: Second target nucleic acid
- 23: Second target's F-primer
- 24: Second target's R-primer
- 25: Second target's probe
- 26: Second labeling substance
- 10 30: DNA polymerase
- 31: Deoxyribonucleoside triphosphate
- T0: Denaturation temperature
- T1: Annealing temperature
- T2: Elongation temperature
- 15 T3: Second target detection temperature

CLAIMS:

1. A kit for together detecting multiple target nucleic acids differing from each other, the kit comprising:

5 solution, the multiple target nucleic acids including a first target nucleic acid and a second target nucleic acid;

 the solution being capable of containing the first target nucleic acid and the second target nucleic acid therein, at a denaturation temperature T0, first double-stranded hydrogen bond of the first target nucleic acid being cut off to be dissociate into first two single strands, second double-stranded hydrogen bond of the second target nucleic acid being cut off to be dissociate into second two single strands, respectively,

 the solution further containing therein:

15 a first target's primer at an annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated;

 a second target's primer at the annealing temperature T1 specifically bonding with either of the second two single strands into which the second target nucleic acid has been dissociated;

20 a first target's probe at the annealing temperature T1 specifically bonding with either of the first two single strands into which the first target nucleic acid has been dissociated, the first target's probe including a first labeling substance changing first fluorescent signals thereof when the first target's probe specifically bonds with either of the first two single strands;

 DNA polymerase;

25 deoxyribonucleoside triphosphate at an elongation temperature T2 bonding by action of the DNA polymerase with both of the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into

which the second target nucleic acid has been dissociated; and

a second target's probe at the annealing temperature T1 bonding with neither the first two single strands into which the first target nucleic acid has been dissociated nor the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe at a second target detection temperature T3 bonding with the second two single strands into which the second target nucleic acid has been dissociated, the second target's probe including a second labeling substance changing second fluorescent signals thereof when the second target's probe specifically bonds with either of the second two single strands,

characterized in that:

T0 is defined as the denaturation temperature; T1 is defined as the annealing temperature; T2 is defined as the elongation temperature; and T3 is defined as the second target detection temperature, T0 through T3 being set up such that a condition that: $T0 > T2 \geq T1 > T3$ is satisfied; and

the first labeling substance is equal to the second labeling substance so that the first fluorescent signals are also equal to the second fluorescent signals.

2. The kit for together detecting multiple target nucleic acids differing from each other as defined in claim 1, wherein the fluorescent signals are shown by quenching light when the annealing occurs.

3. The kit for together detecting multiple target nucleic acids differing from each other as defined in claim 1, wherein the fluorescent signals are shown by emitting light when the annealing occurs.

4. The kit for together detecting multiple target nucleic acids differing from each other as defined in any one of claim 1 to 3, wherein the first target nucleic acid is a

Mycoplasma pneumoniae P1 gene and the second target nucleic acid is internal control composition.

5 5. The kit for together detecting multiple target nucleic acids differing from each other as defined in any one of claim 1 to 3, wherein the first target nucleic acid is a *Chlamydia trachomatis* endogeneous plasmid gene and the second target nucleic acid is a *Nisseria gonorrhoeae* CMT gene.

6. A detection method, comprising:

10 using the kit for together detecting multiple target nucleic acids differing from each other as defined in any one of claim 1 to 5;

15 a dissociating step of increasing temperature of the solution to the denaturation temperature T0 so as to dissociate the first target nucleic acid into the first two single strands, and to dissociate the second target nucleic acid into the second two single strands respectively;

20 a first target nucleic acid detecting step of decreasing the temperature of the solution to the annealing temperature T1 so as to detect whether or not the first target nucleic acid is contained in the solution based on the first fluorescent signals shown by the first target's probe;

25 an amplification step of setting up the temperature of the solution to the elongation temperature T2 so as to amplify the first two single strands into which the first target nucleic acid has been dissociated and the second two single strands into which the second target nucleic acid has been dissociated, respectively; and

30 a second target nucleic acid detecting step of decreasing the temperature of the solution to the second target detection temperature T3 so as to detect whether or not the second target nucleic acid is contained in the solution based on the second fluorescent signals shown by the second target's probe.