PRODUCT INFORMATION

Product Name : PCRChecker
Code No. : DC101
Size : 500 µl × 2 (for 20 reactions) *This product is research use only*

Description :

The polymerase chain reaction (PCR) is the experimental technique which is widely used and indispensable in many life-science research laboratories. Although PCR is an efficient and effective technique, sometimes it gives us troublesome results. In some cases, no amplification occurs. The reason cannot be figured out easily. It may be an error of a thermal cycler, forgetting to put requisite reagents in or using the wrong reagents. The PCRChecker is a versatile positive control for PCR. It can be used as a positive control for broad range PCR conditions, for instance, in multiple denaturing temperatures, wide range of annealing temperatures and various extension times. PCRChecker informs errors of a thermal cycler, nonviable wells in the thermal cycler, or wrong operations of PCR.

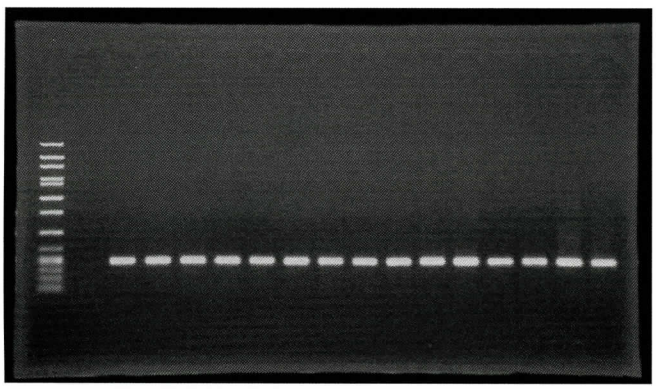

Fig. 1. Electrophoresis profile of PCRChecker in broad range PCR conditions

Electrophoresis was done on a 0.8% agarose gel in 1 × TAE buffer. Details of PCR conditions are described on the next page.

Lane 1: DynaMarker for Plasmid D
Lane 2: PCRChecker, no-amplification control
Lane 3: Annealing at 45°C
Lane 4: Annealing at 50°C
Lane 5: Annealing at 55°C
Lane 6: Annealing at 60°C
Lane 7: Annealing at 65°C
Lane 8: Annealing at 68°C
Lane 9: Extention at 62°C
Lane 10: Extention at 75°C
Lane 11: Extention for 2 min
Lane 12: Extention for 5 min
Lane 13: Extention for 10 min
Lane 14: Long time denature
Lane 15: Annealing for 15 sec
Lane 16: Two-step PCR
Lane 17: Short time denature at 95°C

Storage condition :

Store PCRChecker by freezing (less than -20 °C). The frozen PCRChecker is stable for 6 months from the date of receipt. The PCRChecker withstands up to 3 or 4 times freezing and thawing. If freezing and thawing is expected many times, dispensing aliquots of PCRChecker is recommended.

Acceptable PCR conditions :

PCRChecker generates a 500-bp PCR product on broad range PCR conditions as follow:

Initial Heating:	• 94-95°C, for 30 sec to 15 min • 97-98°C, for 10 sec	• 96°C, for 10 sec to 2 min
Denaturing step:	• 94-95°C, for 20 sec to 1 min • 97-98°C, for 10 sec	• 96°C, for 10 sec to 30 sec
Annealing step:	• 45-68°C, for 15 sec to 2 min	
Extension step:	• 60-75°C, for 30 sec to 15 min	
Cycle #:	• from 15 to 30	

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How to use :

1. As positive control

PCRChecker is an ideal product for a positive control on PCR. PCRChecker works well in broad range PCR conditions. So put a tube containing PCRChecker into the thermal cycler which contains your PCR samples.

1) Put the 50-µl* PCRChecker in a PCR tube.
2) Set your PCR sample(s) in a thermal cycler.
3) Set the PCR tube containing PCRChecker in the same thermal cycler as above.
4) Run PCR according to your PCR cycle. (Acceptable PCR conditions for PCRChecker are above.)
5) Electrophoreses the PCR products (0.5-2 µl, depending on the cycle# and the well size) on agarose gel.

*The volume is important as positive control, less than 50 µl or over 50 µl may not be appropriate.

2. Examine a thermal cycler

Some wells on a thermal cycler may not be controlled well. If such a concern occurs, PCRChecker is a good tool to check the thermal cycler.

1) Put the 50-µl PCRChecker into a PCR tube.
2) Set the PCR tube containing PCRChecker in all wells on the thermal cycler to be checked.
3) Run PCR according to standard PCR cycle* as below:

Initial Heating	95 °C for 2.5 min
25 Cycling	95°C for 30 sec →55 °C for 30 sec→ 72°C for 1 min
Final Extension	72 °C for 5 min

PCR cycle* can be changed within the range of acceptable PCR conditions for PCRChecker.

Cautionary note:

• PCRChecker indicates the possibility of defects on the thermal cycler but does not indicate definitive conclusions.

• As PCR is extreme sensitive technique, adequate care must be taken to avoid contamination with amplified DNA products.

Detail cycle conditions in Fig. 1

Lane	Initial Heating	Cycle	Cycle#
3	95 °C for 2.5 min	95 °C for 30 sec → 45 °C for 30 sec → 72 °C for 30 sec	25
4	95 °C for 2.5 min	95 °C for 30 sec → 50 °C for 30 sec → 72 °C for 30 sec	25
5	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec → 72 °C for 30 sec	25
6	95 °C for 2.5 min	95 °C for 30 sec → 60 °C for 30 sec → 72 °C for 30 sec	25
7	95 °C for 2.5 min	95 °C for 30 sec → 65 °C for 30 sec → 72 °C for 30 sec	25
8	95 °C for 2.5 min	95 °C for 30 sec → 68 °C for 30 sec → 72 °C for 30 sec	25
9	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec → 62 °C for 30 sec	25
10	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec → 75°C for 30 sec	25
11	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec →72 °C for 2 min	25
12	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec →72 °C for 5 min	25
13	95 °C for 2.5 min	95 °C for 30 sec → 55 °C for 30 sec →72 °C for 10 min	25
14	95 °C for 15 min	94 °C for 30 sec → 55 °C for 30 sec → 72°C for 1 min	25
15	none	96 °C for 30 sec → 50 °C for 15 se c→ 60 °C for 4 min	25
16	none	98 °C for 10 sec → 68°C for 10 min	30
17	95 °C for 2 min	95 °C for 15 sec → 55 °C for 30 sec→ 68 °C for 3 min	25

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