PCR Amplification Kit

Store at -20°C

Description

The PCR Amplification kit contains Omega Bio_Tek Taq DNA polymerase, 10 mM dNTPs, 5×Direct-Loading PCR Buffer, 10×PCR Buffer with Mg^{2+}, 10×PCR Buffer without Mg^{2+}, 20 mM $MgCl_2$, 6×Loading Buffer and so on. The 5×Direct-Loading PCR Buffer contains a blue dye that it is to monitor migration progress during electrophoresis. The 10×PCR Buffer without Mg^{2+} allowing users easy optimization in amplification reactions.

Kit components

Cat.No.	TQ2104-01	TQ2104-02	TQ2104-03
Taq DNA Polymerase	500 Units	1000 Units	5000 Units
10 mM dNTPs	250 µl	500 µl	2.5 ml
5×Direct-Loading PCR Buffer	3 X 1ml	6 X 1 ml	30 ml
10×PCR Buffer with Mg^{2+}	1.5 ml	2 X 1.5 ml	10 ml
10×PCR Buffer without Mg^{2+}	1.5 ml	2 X 1.5 ml	10 ml
20 mM $MgCl_2$	0.5 ml	0.5 ml	1 ml
ddH_2O	2 X 1 ml	4 X 1 ml	10 ml
6×Loading Buffer	1 ml	2 X 1 ml	5 ml

Enzyme Concentration

5 U/µl

Magnesium Chloride Solution, 20 mM

Provided to allow users to optimiize $MgCl_2$ concentration according to their individual requirements. **Vortex the $MgCl_2$ thoroughly after thawing and prior to use.**

Quality Control Assays

No contaminating endonuclease or exonuclease activity detected.

The 5×Direct-Loading PCR Buffer, 10×PCR Buffer with Mg^{2+}, 10×PCR Buffer without Mg^{2+} add 20 mM $MgCl_2$, are tested for performance in the PCR using 2 units Omega Bio_Tek Taq DNA polymerase to amplify a 600 bp region of the 18S rRNA gen from 0.1 µg of mouse genomic DNA. The resulting PCR product is visualized as a singale band on an ethidium bromide-stained 1% agarose gel.

Protocol

1. In a sterile, nuclease-free microcentrifuge tube, combine the following on ice:

a. by 5×Direct-Loading PCR Buffer reaction

Component	Volume
5×Direct-Loading PCR Buffer	10µl
Template DNA(＜0.5 µg)	xµl
Sense primer(10 µM)	1µl
Anti-sense primer(10 µM)	1µl
Bio_Tek Taq DNA polymerase（5 U/µl）	0.25-0.5µl
dNTPs（10 mM）	1µl
Nuclease-free water	Up to 50µl

b. by 10×PCR Buffer with Mg^{2+} reaction

Component	Volume
10×PCR Buffer with Mg^{2+}	5µl
Template DNA(＜0.5 µg)	xµl
Sense primer(10 µM)	1µl
Anti-sense primer(10 µM)	1µl
Bio_Tek Taq DNA polymerase（5 U/µl）	0.25-0.5µl
dNTPs（10 mM）	1µl
Nuclease-free water	Up to 50µl

c. by 10×PCR Buffer without Mg^{2+} reaction

Component	Volume
10×PCR Buffer without Mg^{2+}	5µl
$MgCl_2$ Solution，20 mM	2.5-10µl (1-4 mM)
Template DNA(＜0.5 µg)	xµl
Sense primer(10 µM)	1µl
Anti-sense primer(10 µM)	1µl
Bio_Tek Taq DNA polymerase（5 U/µl）	0.25-0.5µl
dNTPs（10 mM）	1µl
Nuclease-free water	Up to 50µl

Note: thaw completely and vortex thoroughly prior to use.

2. Perform PCR using your standard parameters. An example profile is given in above tables. For the cycling protocol, we recommend the following:

Denaturation	1 Cycle	94°C for 2-5 min
PCR amplification	25-35 Cycles	94°C for 30-60 s 50-60°C for 30 s 72°C for 1 min/kb
Final extension	1 Cycle	72°C for 5 min
End	Hold	4°C

Note: if using a thermal cycler without a heated lid, overlay the reaction mix with 1-2 drops of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.

3. Separate the PCR products by agarose gel electrophoresis and visualize with ethidium bromide or any other means. For reactions containing the 5×Direct-Loading PCR Buffer , load the reaction onto the gel directly after amplification. Reactions containing the 10×PCR Buffer with Mg^{2+} or 10×PCR Buffer without Mg^{2+} will need to add 6×loading buffer to electrophoresis.

The above cycling conditions were established and tested using a MJ Rearch PTC-200 .You may need to adjust these conditions for other thermal cyclers.

Ordering Information

Cat.No.	**TQ2104-01**	**TQ2104-02**	**TQ2104-03**
PCR Amplification kit	500 units	1000 units	5000 units