Certificate of Analysis

PCR Nucleotide Mix:

Part No.	Size
C114G	200µl
C114H	1,000µl

Description: PCR Nucleotide Mix is a premixed solution containing the sodium salts of dATP, dCTP, dGTP and dTTP, each at a concentration of 10mM in water; the total concentration of nucleotides, therefore, is 40mM (pH 7.5). This solution is ready to use and is optimized for standard polymerase chain reactions and specialty approaches including hot-start and reverse transcription PCR (RT-PCR).

Storage Conditions: See the Product Information Label for storage recommendations. Avoid exposure to frequent temperature changes. Mix well prior to use.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assays

Activity Assays

DNase and RNase Assay: To test for nuclease activity, 50ng of [^{3}H]DNA or 50ng of [^{3}H]RNA is incubated with 5µl of PCR Nucleotide Mix for 2 hours at 37°C in 1X MULTI-CORE™ Buffer (Cat.# R9991). The release of radiolabeled nucleotides is measured by liquid scintillation counting of TCA-soluble material. The specification is <3% release.

Physical Purity: The individual dNTPs contained in the PCR Nucleotide Mix each contain ≥99.0% triphosphate as measured by HPLC.

PCR Functional Assay: The individual dNTPs contained in the PCR Nucleotide Mix are tested using PCR to amplify a 360bp region of the α-1-antitrypsin gene. The amplification product is detected as a single 360bp band following agarose gel electrophoresis and ethidium bromide staining.

RT-PCR Functional Assay: The PCR Nucleotide mix is tested using RT_PCR to produce a 323bp amplimer from 0.25 zeptomoles of starting RNA template (100 copies).

PCR Nucleotide Mix

REF C1141 -30°C -10°C

LOT 0000211002
2019-04-25
Dispensed Lot#: 0000202721

200µl

For Laboratory Use

Country of Origin: USA

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711-5399 USA

ADC1141 00002110027

PEEL HERE

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Signed by: J. Stevens

J. Stevens, Quality Assurance

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Promega

Usage Information

1. Description

Applications of PCR Nucleotide Mix that have been tested include:

- PCR amplification by standard approaches.
- PCR amplification by specialty approaches such as hot-start and RT-PCR.

PCR Nucleotide Mix may be applicable to other specialty PCR approaches such as high-fidelity and long PCR.

2. Standard Applications

Preparing the Reaction Mix for PCR Using *Taq* DNA Polymerase

Reagents to Be Supplied by the User

(Solution compositions are provided in Section IV.)

- $MgCl_2$, 25mM (Cat.# A3511, A3512 or A3513)
- Nuclease-Free Water (Cat.# P1193)
- *Taq* DNA polymerase, 5u/µl
- 10X reaction buffer with 15mM $MgCl_2$
- 10X reaction buffer without $MgCl_2$ (optional)
- upstream and downstream primers, each at 20µM

1. Completely thaw, vortex and centrifuge all reagents before beginning the procedure.

   Note: Optimal conditions, including reaction times, temperatures and reagent concentrations are dependent on the *Taq* DNA polymerase, template and primers used. The **magnesium concentration is especially important** and should be titrated between the range of 1.5–3.0mM in order to ensure optimal results. In many cases, a magnesium concentration of 1.5mM will result in satisfactory amplification. Therefore, two reaction preparations are given below. The first reaction is for 10X reaction buffer with 15mM $MgCl_2$. The second reaction is for 10X reaction buffer **without** 15mM $MgCl_2$.

2. Prepare one of the reaction mixtures listed below by adding reagents to a sterile microcentrifuge tube in the specified order.

   a. Reaction components for **10X reaction buffer with 15mM $MgCl_2$**:

Component	Component Volumes	Final Concentration
10X reaction buffer (with 15mM $MgCl_2$)	5µl	1X
PCR Nucleotide Mix (10mM each dNTP)	1µl	*800µM
upstream primer, 20µM	0.25–2.5µl	0.1–1µM
downstream primer, 20µM	0.25–2.5µl	0.1–1µM
Taq DNA polymerase, 5u/µl	0.25µl	0.025u/µl
template DNA	Xµl	<250ng
Nuclease-Free Water to **final volume** of	**50µl**	

   b. Reaction components for **10X reaction buffer without 15mM $MgCl_2$**:

Component	Component Volumes	Final Concentration
$MgCl_2$, 25mM	3µl	1.5mM
10X reaction buffer (without 15mM $MgCl_2$)	5µl	1X
PCR Nucleotide Mix (10mM each dNTP)	1µl	*800µM
upstream primer, 20µM	0.25–2.5µl	0.1–1µM
downstream primer, 20µM	0.25–2.5µl	0.1–1µM
Taq DNA polymerase, 5u/µl	0.25µl	0.025u/µl
template DNA	Xµl	<250ng
Nuclease-Free Water to **final volume** of	**50µl**	

*The final concentration of each dNTP is 200µM.

3. Vortex the mixture and centrifuge briefly to ensure all liquid is at the bottom of the tube.
4. If using a thermal cycler without a heated lid, overlay the surface of the reaction mixture with 25–50µl of mineral oil.
5. Place the reaction tubes in a thermal cycler. The times and temperatures for denaturation, annealing and extension phases of amplification are template- and primer-dependent. Follow the general amplification guidelines in Section III as a starting point.

3. General Guidelines for Amplification by PCR

The following guidelines apply to target sequences between 200 and 2,000bp and are optimal for the Perkin-Elmer Thermal Cycler Model 480 or comparable thermal cyclers.

Note: Optimal denaturation and annealing reaction times for the Perkin-Elmer Thermal Cycler Model 9600, or comparable thermal cyclers, are shorter.

A. Denaturation

- Generally, a 2-minute initial denaturation step at 95°C is sufficient.
- Subsequent denaturation steps will be between 30 seconds and 1 minute.

B. Annealing

- Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.
- The annealing step is typically 30 seconds to 1 minute.

C. Extension

- The extension reaction is typically performed at the optimal temperature for *Taq* DNA Polymerase, which is 72–74°C.
- Allow approximately 1 minute for every 1kb of DNA to be amplified.
- Minimum extension time should be 1 minute.
- A final extension of 5 minutes at 72–74°C is recommended.

D. Soaking

- If the thermal cycler has a "soak" cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.
- This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

- Generally, 25–30 cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially for detection of low-copy message.

4. Composition of Buffers and Solutions

10X reaction buffer with 15mM $MgCl_2$

100mM	Tris-HCl (pH 9.0 at 25°C)
500mM	KCl
15mM	$MgCl_2$
1%	Triton® X-100

10X reaction buffer without 15mM $MgCl_2$

100mM	Tris-HCl (pH 9.0 at 25°C)
500mM	KCl
1%	Triton® X-100

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