Certificate of Analysis

Part# 9PIC114

Revised 10/11

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).

Promega

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SATISFACTION PCR GUARANTEE

PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

Signed by: J. Stevens

J. Stevens, Quality Assurance

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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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