TruePrime®
RCA Kit

HANDBOOK

4basebio

INDEX

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
TruePrime® RCA kit	100 reactions	390100

KIT CONTENTS

DESCRIPTION	CAP COLOR	REACTIONS
Buffer D	Red	1 x 275 µl
Buffer N	Translucent	1 x 275 µl
Reaction Buffer	Yellow	1 x 550 µl
dNTPs	Green	1 x 275 µl
H_2O	Blue	1 x 1500 µl
Enzyme 1	Purple	1 x 280 µl
Enzyme 2	Orange	1 x 80 µl

Buffer D: denaturing buffer; Buffer N: neutralization buffer; Enzyme 1: *TthPrimPol*; Enzyme 2: Phi29 DNA polymerase.

SHIPPING AND STORAGE

TruePrime® RCA Kit is shipped in dry ice. Upon receipt, the kit should be stored immediately at –20ºC in a non-frost-free (constant temperature) freezer. If stored correctly, the product can be kept for at least 24 months after shipping, without displaying any reduction in performance. For longer period, we recommend, store the kit at -80ºC.

HANDLING

This kit is sensitive to small amounts of DNA. Wear gloves at all times and prepare the reaction in a laminar flow hood or similar device to avoid contaminations. Use molecular biology grade clean reagents, sterile reaction tubes and DNA-free pipette tips. Thaw Enzyme 1, Enzyme 2 and dNTPs on ice. All other components can be thawed at room temperature.

All chemicals should be considered as potentially hazardous. This material may contain substances or activities that are harmful to human health. It should not be ingested, inhaled, or brought into contact with skin, and handled with appropriate care in accordance with the principles of good laboratory practice.

In case of contact with skin wash immediately with water. Buffer D contains potassium hydroxide, which is corrosive and harmful. Risk and safety phrases: R22-35. S26-36/37/39-45. For more specific information please consult the Material Safety Data Sheets (MSDS) available on-line at www.4basebio.com.

QUALITY CONTROL

Each batch of TruePrime® RCA Kit is tested against predetermined specifications to ensure consistent product quality. Enzymes used in the kit have been tested separately to ensure adherence to specifications.

REAGENTS AND EQUIPMENT TO BE SUPPLIED BY THE USER

- Sterile vials, pipettes and pipette tips
- Microcentrifuge
- Cold block
- Sterile, ideally DNA-free certified 0.2 ml PCR tubes
- Thermocycler
- Vortexer
- 1 x TE buffer
- Optional: Quant-iT™ Picogreen® dsDNA quantification reagent (Invitrogen, P7581) or similar reagents

INTRODUCTION

TruePrime® RCA Kit uses a novel multiple displacement amplification method based on the combination of the recently discovered DNA primase *TthPrimPol* and the highly processive and high-fidelity Phi29 DNA polymerase to amplify single- or double-stranded circular DNA molecules by rolling circle amplification (RCA). The strong strand displacement capacity of Phi29 DNA polymerase allows *TthPrimPol* to generate new primers on the displaced strands that are extended by Phi29 DNA pol, resulting in exponential isothermal DNA amplification. TruePrime® RCA Kit eliminates the need for overnight cell culture and conventional DNA purification.

The starting material for TruePrime® rolling circle amplification can be:

- Purified DNA: plasmids, cosmids, BACs, M13 clones, etc.
- Bacterial cells: colonies picked from agar plates, bacterial cultures or glycerol stocks

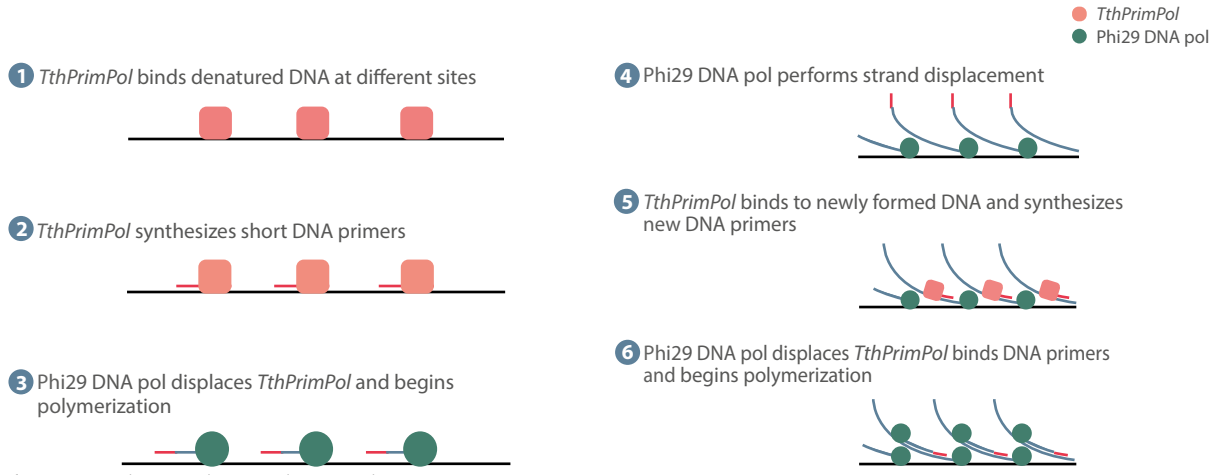

Figure 1. Overview over the TruePrime® reaction

PROTOCOL

TruePrime® RCA Kit uses a novel and reliable method to achieve accurate amplification of single- or double-stranded circular DNA molecules. Dedicated buffers and enzymes deliver microgram quantities of DNA.

Typical DNA yields from a TruePrime® RCA Kit reaction are above 3 µg per 25 µl reaction and 3-hour reaction time when starting from 1 ng of plasmid DNA (3-10 kb). For bigger template molecules (150-200 kb) yields are approximately 2 µg per 25 µl reaction and 3-hour reaction time when starting from 10 ng. Yields and kinetics will vary if crude or un-quantified samples are amplified.

Reactions without input DNA (no template controls) do not produce any amplification product during 3-hour reaction times. The product of TruePrime® RCA reaction is high molecular weight, double-stranded concatemers of the circular template. Amplification products can be transformed after digestion with a singlesite restriction enzyme and re-ligation. Store amplified DNA at 4°C for short-term storage or at -20°C for long-term storage.

TruePrime® RCA Kit uses alkaline incubation to allow DNA release and denaturation with very low DNA fragmentation. This results in amplified DNA with high integrity and fragment length.

A. Short Protocol

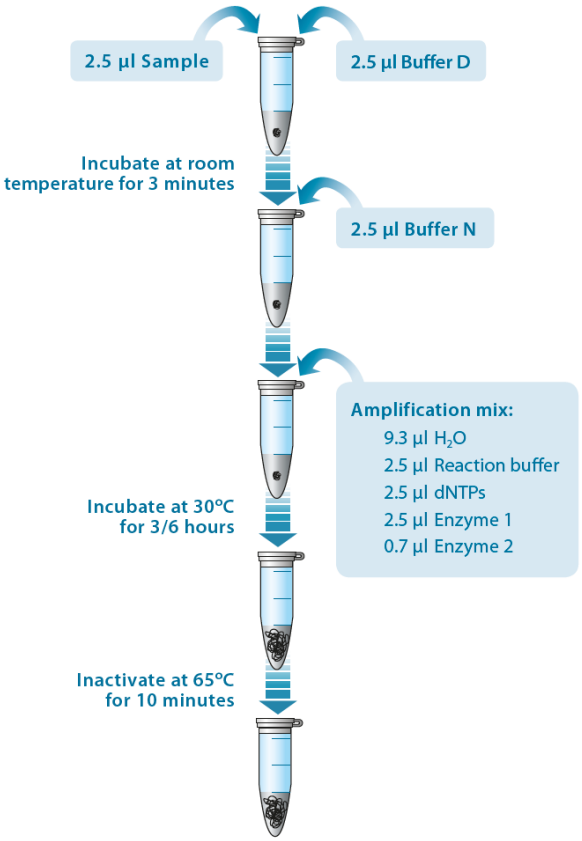

Figure 2. Schematic representation of TruePrime® RCA Kit protocol

B. Detailed Protocol

The protocol described below is a general protocol for amplifying circular DNA molecules. It should be considered a starting point for adapting your specific reaction.

B.1. Sample prep for purified DNA:

- Always mix by pipetting. DO NOT VORTEX
- Transfer 2.5 µl of circular DNA (≥ 400 fg/µl) into a 0.2 ml PCR tube
- Add 2.5 µl of Buffer D and incubate at room temperature for 3 minutes
- Neutralize the reaction by adding 2.5 µl of Buffer N to each tube
- Keep the samples at room temperature until use*

B.2. Sample prep for bacterial cells:

B.2.A Bacterial colonies:

- Resuspend a colony in 10 µl of 1x TE
- Transfer 2.5 µl into a 0.2 ml PCR tube
- Add 2.5 µl of Buffer D and incubate at room temperature for 3 minutes
- Neutralize the reaction by adding 2.5 µl of Buffer N to each tube
- Keep the samples at room temperature until use*

B.2.B Liquid bacterial culture:

- Transfer 2.5 µl of bacterial culture into a 0.2 ml PCR tube
- Add 2.5 µl of Buffer D and incubate at room temperature for 3 minutes
- Neutralize the reaction by adding 2.5 µl of Buffer N to each tube
- Keep the samples at room temperature until use*

B.2.C Glycerol stock:

- Dilute 1 µl of glycerol stock in 9 µl of 1x TE
- Transfer 2.5 µl into a 0.2 ml PCR tube
- Add 2.5 µl of Buffer D and incubate at room temperature for 3 minutes
- Neutralize the reaction by adding 2.5 µl of Buffer N to each tube
- Keep the samples at room temperature until use*

B.3. Amplification reaction:

Prepare the amplification mix adding the components in the order listed in the following table:

COMPONENT	VOLUME
H_2O	9.3 µl
Reaction Buffer	2.5 µl
dNTPs	2.5 µl
Enzyme 1	2.5 µl
Enzyme 2	0.7 µl

Table 1. Preparation of amplification mix

Note: Scale up accordingly (10% excess recommended) when performing several reactions at the same time. Mix the amplification mix by vortexing and add 17.5 µl to each sample (7.5 µl).

Incubate at 30°C for 3 hours**. Inactivate the reaction at 65°C for 10 minutes.

Cool down to 4°C. Store amplified DNA at 4°C for short-term storage or -20°C for long-term storage.

(*) It is highly recommended to perform the amplification reaction just after the sample has been denatured.
(**) Incubation time can be increased up to 6 hours if higher amplification yields are required.

QUANTIFICATION OF TRUEPRIME® AMPLIFIED DNA

This protocol is designed for quantification of double stranded TruePrime® amplified DNA using PicoGreen® reagent.

1. Make a 1:150 dilution of PicoGreen® stock solution in 1x TE (10 mM Tris-HCl pH 8; 1 mM EDTA). Each quantification reaction requires 20 µl. Example: for 30 measurements add 4 µl of PicoGreen® to 596 µl 1x TE. Protect the solution from light at all times to avoid photodegradation of the PicoGreen® reagent.
2. Prepare a standard curve using genomic DNA. Prepare a 16 µg/ml stock solution of gDNA in 1x TE buffer.
3. Prepare 200 µl of 1.6, 0.8, 0.4, 0.2 and 0.1 µg/ml of genomic DNA using 1x TE.
4. Transfer 20 µl of each DNA standard in duplicate into a 96-well plate labelled A.
5. Dilute each amplified DNA sample 1:100 (2 µl sample + 198 µl 1x TE) in a 96- well plate labelled B.
6. Place 2 µl of the 1:100 DNA sample dilution into the 96-well-plate labelled A and add 18 µl of 1x TE (dilution 1:1000). Residual 1:100 dilution (plate labelled B) might be stored at -20°C for further analysis.
7. Add 20 µl of PicoGreen® dilution to each sample (amplified DNAs and DNA standards) in the 96-well-plate labelled A. Gently shake the plate to mix the samples and reagent.
8. Measure fluorescence in a microplate reader (excitation wavelength ≈480nm, emission wavelength ≈520nm).
9. Calculate the concentration of the amplification product: Generate a standard curve of fluorescence versus concentration of DNA standards. Determine the concentration of TruePrime® amplified products from the equation of the line derived from the standard curve. Dilution factor during the assay must be taken into consideration when calculating total yields.

TROUBLESHOOTING GUIDE

REASON	SOLUTION
Reduced yield or no amplification product	**Contamination of template DNA** Use sterile laboratory equipment and barrier pipette tips. Work in a laminar-flow hood. Use molecular biology grade PBS, TE and water to prepare all samples. **Carryover of alcohol in isolated DNA sample** When using column-based purification procedures, ensure the duration of the drying step prior to elution of DNA from the column is sufficient to evaporate residual ethanol. **Low quality DNA** Avoid template preparation steps that can damage DNA. **Inactive Enzymes** Enzyme 1 and Enzyme 2 should be properly stored at -20°C. The freezer must be a non-frost-free (constant-temperature) freezer. **Prolonged DNA denaturation** Avoid incubation periods longer than 3 minutes at room temperature because it may nick the DNA template and decrease the amplification efficiency.
Poor performance in downstream applications	**Degraded or low amounts of template DNA** Use high quality genomic DNA for amplification. **Presence of non-specific amplification product** Use sterile laboratory equipment and barrier pipette tips. Work in a laminar-flow hood. Use molecular biology grade PBS, TE and water to prepare all samples.

4basebio

orders@4basebio.com

www.4basebio.com