TruePrime™ LB
Necrotic cell-free/exosomal DNA amplification kit

expedeon

INDEX

Ordering Information 3
Kit Contents 3
Shipping and Storage 3
Handling 3
Quality Control 3
Reagents and Equipment to be Supplied by the User 4
Introduction 4
Protocol 4
PicoGreen® Quantification of TruePrime™ Amplified DNA 6
Qubit™ Quantification of TruePrime™ Amplified DNA 7
Troubleshooting Guide 8

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
TruePrime™ Necrotic cell-free/exosomal DNA amplification kit	25 reactions	330025

KIT CONTENTS

DESCRIPTION	CAP COLOR	REACTIONS
Buffer D	Red	1 x 70 µl
Buffer N	Translucent	1 x 70 µl
Reaction Buffer	Yellow	1 x 140 µl
dNTPs	Green	1 x 140 µl
H_2O	Blue	1 x 750 µl
Enzyme 1	Purple	1 x 140 µl
Enzyme 2	Orange	1 x 20 µl

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

SHIPPING AND STORAGE

TruePrime™ Necrotic cell-free/exosomal DNA amplification kit is shipped in dry ice. Upon receipt, the kit should be stored immediately at -20°C in a non-frost-free (constant-temperature) freezer. If stored correctly, the product can be kept for at least six months after shipping without displaying any reduction in performance. For longer periods, store the kit at -80°C. Avoid repeated freeze-thaw cycles if possible.

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.

Any human body fluids should always be considered as potentially infectious, and appropriate care should be taken when handling these fluids.

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.expedeon.com.

QUALITY CONTROL

Each batch of TruePrime™ Necrotic cell-free/exosomal DNA amplification 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. Use low-retention plasticware if possible.
- Microcentrifuge
- Cold block
- Sterile, ideally DNA-free certified 0.2 ml PCR tubes
- Thermocycler
- Vortexer
- 1 x TE buffer

INTRODUCTION

TruePrime™ Necrotic cell-free/exosomal DNA amplification 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 genomic DNA starting from cell-free DNA obtained from plasma, serum, urine or CSF. 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.

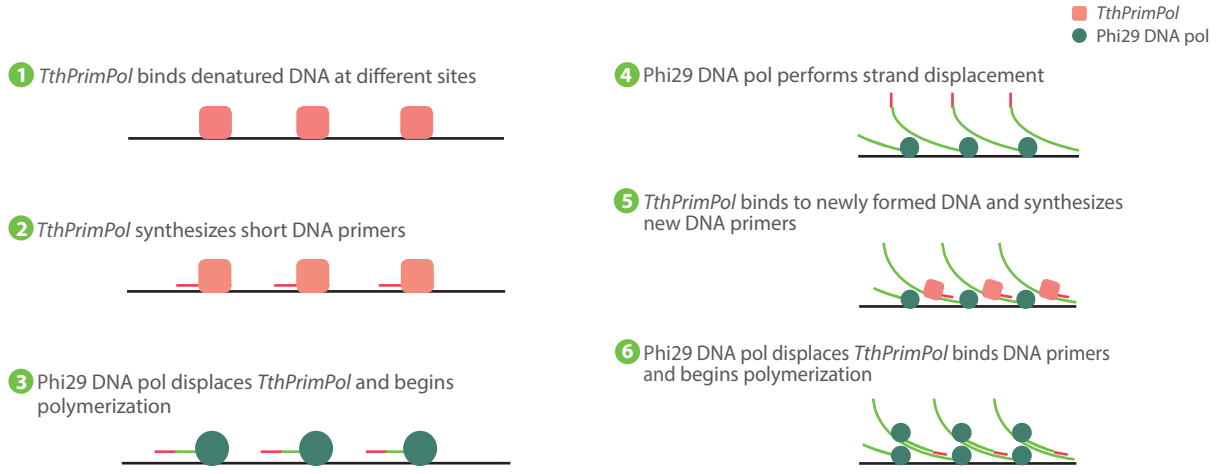

Figure 1. Overview over the TruePrime™ reaction

PROTOCOL

TruePrime™ Necrotic cell-free/exosomal DNA amplification kit uses a novel and reliable method to achieve accurate DNA amplification from cell-free DNA. Dedicated buffers and enzymes deliver microgram quantities of DNA. Typical DNA yields from a TruePrime™ Necrotic cell-free/exosomal DNA amplification kit reaction are about 3 µg per 50 µl reaction and 3 hours reaction time when starting from 1 ng of purified cell-free DNA. Please note that the apparent yield may change depending on the quantification chosen (see also “Expected yield” section). 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. Store amplified DNA at 4°C for short-term storage or -20°C for long-term storage.

A. Short Protocol

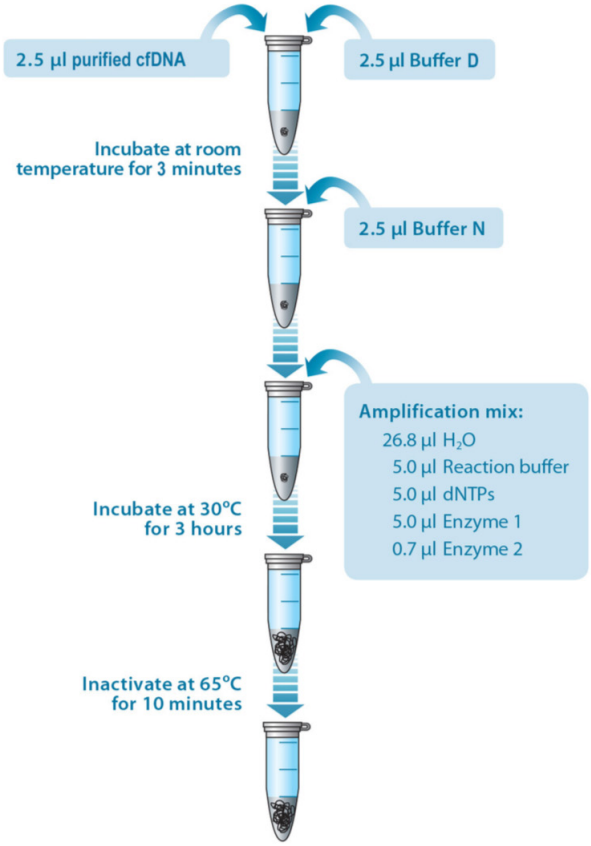

Figure 2. Schematic representation of TruePrime™ Necrotic cell-free/exosomal DNA amplification kit protocol.

B. Detailed Protocol

The protocol described below is a general protocol for amplifying cell-free DNA from purified material or directly from plasma or serum. It should be considered a starting point for adapting your specific reaction.

B.1. Preparation of amplification mix:

Prepare the amplification mix adding the components in the order listed in Table 1.

COMPONENT	VOLUME
H2O	26.8 µl
Reaction Buffer	5 µl
dNTPs	5 µl
Enzyme 1	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 store on ice until use.

B.2. DNA denaturation:

Always mix carefully and spin down when needed. DO NOT VORTEX.

Transfer 2.5 µl of purified cell-free DNA into a 0.2 ml PCR tube (recommended cfDNA concentration: 4-400 pg/µl).

Note: Plasma or serum might be used also as direct input into the reaction. 2.5 µl of plasma/serum diluted 10-fold in TE 1x might be a good initial input. However, the amplification yields will depend on the cfDNA content of each sample, so several crude input doses might be required to reach an efficient amplification. Purified

cfDNA from urine and cerebrospinal fluids could also be entered in the reaction here, although this has not been directly tested by us so far.

Add 2.5 µl of Buffer D and incubate for 3 min at room temperature.

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:

Add 42.5 µl amplification mix to each denatured sample (7.5 µl), mix by pipetting and 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 longterm storage. Incubation time can be increased to 6 hours if higher amplification yields are needed.

Optional: Positive control

Purified genomic DNA can be used as positive control. After 3h of amplification a yield around 3 µg is expected from 100 pg of gDNA input (note: possible differences in apparent yield might depend on the quantification method used).

PICOGREEN® 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.

Qubit™ QUANTIFICATION OF TRUEPRIME™ AMPLIFIED DNA

We recommend the Qubit procedure, as it produces more reliable results in our hands. Please follow the manufacturer's recommendations.

Expected yields

Below you will find the DNA yields obtained after amplifications of cell-free DNA purified from human serum (SIGMA Ref. H3667 Lot SLBM1923V) and plasma (SIGMA Ref. P9523 Lot SLBK0464V) for 3 hours. DNA yield was quantified using PicoGreen® reagent. Apparent yields will on average be about 25% lower with the Qubit™ method, and there is some consensus that the PicoGreen® method overestimates the DNA concentration.

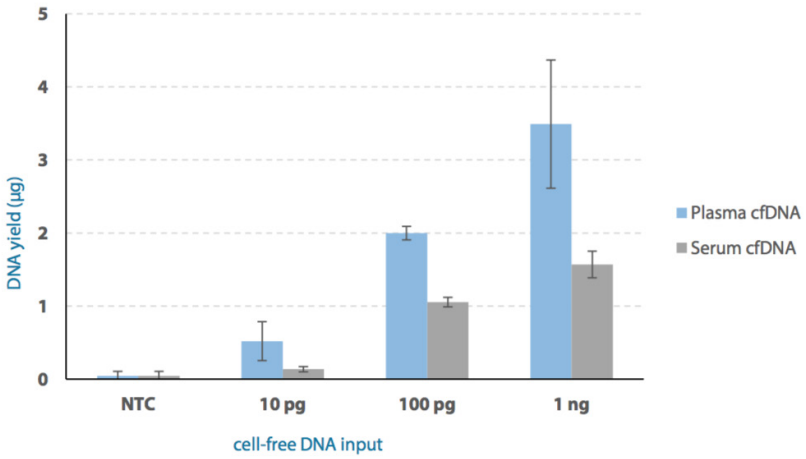

Figure 3. Expected amplification DNA yields depending on the cell-free DNA source (NTC = no template control).

These yields might vary depending on:

- Type of blood collection tube
- Blood storage temperature
- Time between sample collection and plasma/serum preparation
- Time between blood centrifugation and plasma/serum transfer
- DNA purification kit

We recommend the following materials and conditions:

- Collection tube: Regular EDTA tubes (e.g. BD Vacutainer K3 EDTA) or specialized ones like Cell-free DNA BCT (Streck) or PAXgene Blood ccfDNA Tube (PreAnalytix, 768115)
- Time between extraction and plasma/serum preparation: less than 2 hours
- Blood storage temperature: 4°C
- Centrifugation conditions: 4°C, 1500-2000 rcf, double-spin
- Serum/plasma collection: 1.5 ml per 5 ml of blood (first centrifugation) and 1 ml per 1.5 ml of plasma (second centrifugation)
- cfDNA purification: QIAamp Circulating Nucleic Acid Kit (Cat.No. 55114)
- cfDNA quantification: Qubit™ quantification

Some variance between samples is expected depending on the source and status of each sample.

NTC (no template control) or negative controls should not show any DNA amplification up to three hours.

Processing of samples for subsequent PCR

For performing subsequent PCR on TruePrime™ amplification products we highly recommend to purify the samples beforehand as TruePrime™ components can have inhibitory effects on PCR. We recommend the Qiagen Qiaquick PCR purification kit (Cat No./ID: 28104), the Qiagen QIAamp DNA Mini Kit (Cat No./ID: 51304), or ethanol precipitation. All have worked in our hands, with slight advantages for the columns.

Processing of samples for subsequent NGS

The size distribution of the amplification fragments will be approximately between 1 and 20 kb, with a peak around 9 kb. The DNA can be handled in general like non-amplified high molecular weight genomic DNA. For purification we recommend ethanol precipitation and washing with 70% EtOH. Fragmentation of amplified DNA will work well for example with the Covaris sonication fragmentation using parameters suited for extracted genomic DNA. Library construction can be performed with all commonly available construction kits.

Coverage

The completeness of DNA amplification from cell-free DNA may vary, leading to different degrees of coverage breadth in samples processed in parallel. We recommend to either perform low depth NGS, check coverage, and select samples for deeper sequencing based on the results. Another possibility for human samples is to use the Expedeon CovCheck™ kit (Cat. No. CVC0004).

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.
	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	**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.

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