TruePrime™
Single Cell WGA Kit version 2.0

expedeon

INDEX

ORDERING INFORMATION

PRODUCT	SIZE	CAT. NO.
TruePrime™ Single Cell WGA Kit version 2.0	25 reactions	351025
TruePrime™ Single Cell WGA Kit version 2.0	100 reactions	351100

KIT CONTENTS

DESCRIPTION	CAP COLOR	25 REACTIONS	100 REACTIONS
Buffer L1	Red	1 x 70 µl	1 x 250 µl
DTT	White	2 x 50 µl	2 x 50 µl
Buffer N	Translucent	1 x 70 µl	1 x 275 µl
Reaction Buffer	Yellow	1 x 140 µl	1 x 550 µl
dNTPs	Green	1 x 140 µl	1 x 550 µl
H_2O	Blue	1 x 750 µl	2 x 1500 µl
Enzyme 1	Purple	1 x 140 µl	1 x 550 µl
Enzyme 2	Orange	1 x 20 µl	1 x 80 µl
Control 1	Amber	1 x 50 µl	1 x 50 µl
Control 2	Amber	1 x 50 µl	1 x 50 µl

Buffer L1: lysis buffer; Buffer N: neutralization buffer; Enzyme 1: *TthPrimPol*; Enzyme 2: Phi29 DNA polymerase, Control 1/2: human genomic DNA..

SHIPPING AND STORAGE

TruePrime™ Single Cell WGA Kit version 2.0 is shipped on 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.

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 L1 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™ Single Cell WGA Kit version 2.0 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
- 1x PBS buffer
- 1 x TE buffer
- Optional: Quant-iT™ Picogreen® dsDNA quantification reagent (Invitrogen, P7581), Qubit™ dsDNA HS or BR Assay Kit (ThermoFisher, Q32851 or Q32850) or similar reagents

INTRODUCTION

TruePrime™ Single Cell WGA Kit version 2.0 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 total genomic DNA either from a single cell or a few cells. 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.

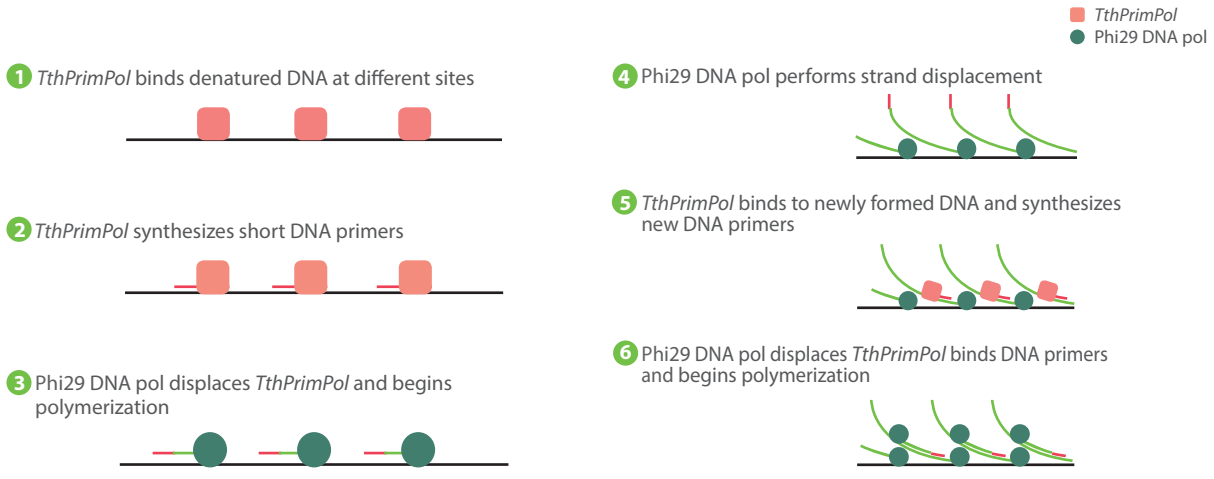

Figure 1. Overview over the TruePrime™ reaction

PROTOCOL

TruePrime™ Single Cell WGA Kit version 2.0 uses a novel and reliable method to achieve accurate genome amplification from single cells. Dedicated buffers and enzymes deliver microgram quantities of DNA from a single cell or few cells. Up to 50 cells can be amplified with the same protocol. Amplification of a few cells instead of one can improve total coverage breadth if your experimental design allows this. Typical DNA yields from a TruePrime™ Single Cell WGA kit version 2.0 reaction are about 3-4 µg per 50 µl reaction and 3 hours reaction time when starting from a single mammalian cell.

Please note that the apparent yield may change depending on the quantification method chosen (see also "Expected yield" section). Yields and kinetics will vary if crude or unquantified samples are amplified. Reactions without input DNA (no template controls) do not produce any amplification product during 3 hour reaction times. Mean product length is greater than 10 kb. Store amplified DNA at 4°C for short-term storage or -20°C for long-term storage.

TruePrime™ Single Cell WGA Kit version 2.0 uses alkaline incubation to allow cell lysis and DNA denaturation of genomic DNA with very low DNA fragmentation. This results in amplified DNA with high integrity and fragment length, so that most of the sequences are uniformly represented.

A. Short Protocol

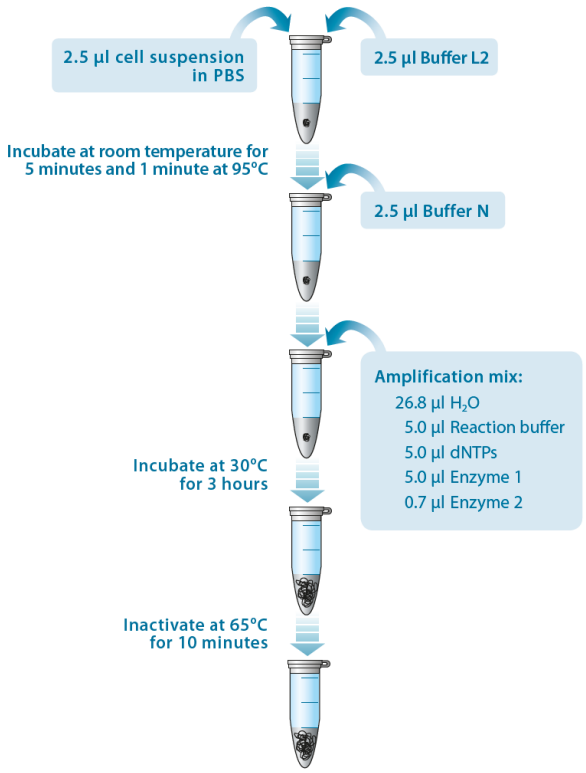

Figure 2. Schematic representation of TruePrime™ Single Cell WGA Kit version 2.0 protocol.

B. Detailed Protocol

The protocol described below is a general protocol for amplifying genomic DNA from single cells. 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 (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. Preparation of lysis buffer:

Prepare enough Buffer L2 (lysis buffer) for the intended the number of whole genome amplification reactions.

COMPONENT	VOLUME
DTT	2.5 µl
Buffer L1	22.5 µl

Table 2. Preparation of Buffer L2

The final volume stated in Table 2 is enough for 10 WGA reactions. We recommend to prepare a 10% excess of volume. Buffer L2 should be prepared freshly for the respective number of reactions.

B.4. Cell lysis:

- Always mix carefully and spin down when needed. DO NOT VORTEX.
- Count cells and dilute with 1x PBS buffer to the required final concentration (0.4-20 cells/µl)
- Transfer 2.5 µl of cell suspension into a 0.2 ml PCR tube
- Add 2.5 µl of Buffer L2 and incubate for 5 min at room temperature and 1 min at 95°C. Place on ice immediately
- Neutralize cell lysis by adding 2.5 µl of Buffer N to each tube
- Keep the samples on ice until use

Note on DTT: Very rarely precipitates have been observed in thawed DTT tubes. If that happens, we recommend to discard the tube and use the second DTT tube (white tube) instead.

B.3. Amplification reaction:

Add 42.5 µl of amplification mix to each sample (7.5 µl cell lysis reaction), 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 long-term storage. Incubation time can be increased to 6 hours if higher amplification yields are needed.

Optional: Positive control

Control 1 and Control 2 contain purified human genomic DNA at 40 pg /µl and 4 pg/µl, respectively. Ideally run the positive controls in parallel, using 2.5 µl of Control 1 and/or 2 instead of the cell suspension. This will result in an amount of 100 and 10 pg genomic DNA input, roughly equivalent to the genomic DNA content of 15 or 1.5 human cells. We recommend to use Control 2 (10 pg input) as the positive control for single cell experiments, and Control 1 (100 pg) for experiments with more than one cell. After 3h of amplification a yield around 3 µg is expected from Control 1 (check Figure 3) (note possible differences in apparent yield depending on the quantification methods).

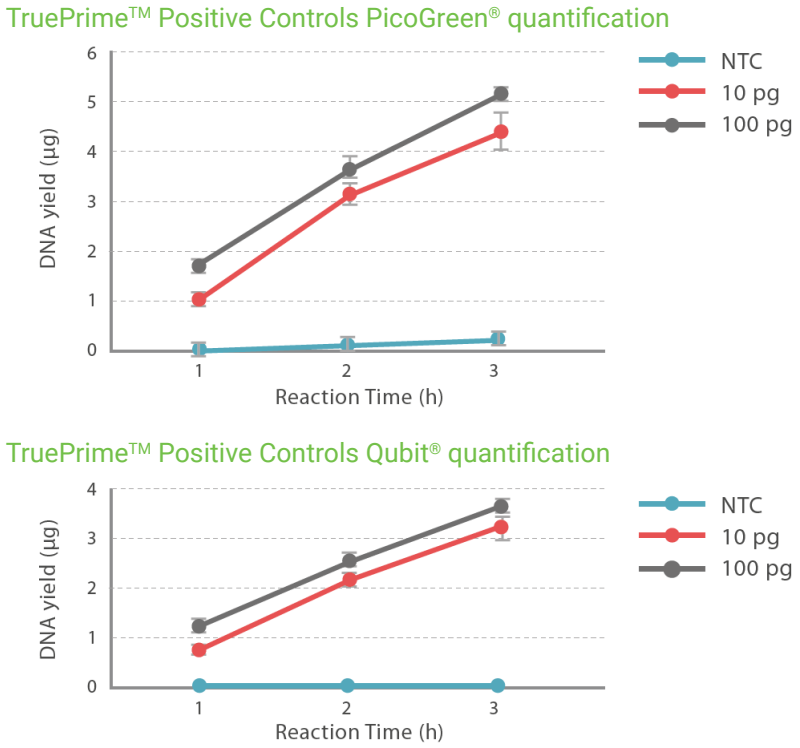

Figure 3. Expected yields from positive controls depending on the quantification method used.

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.

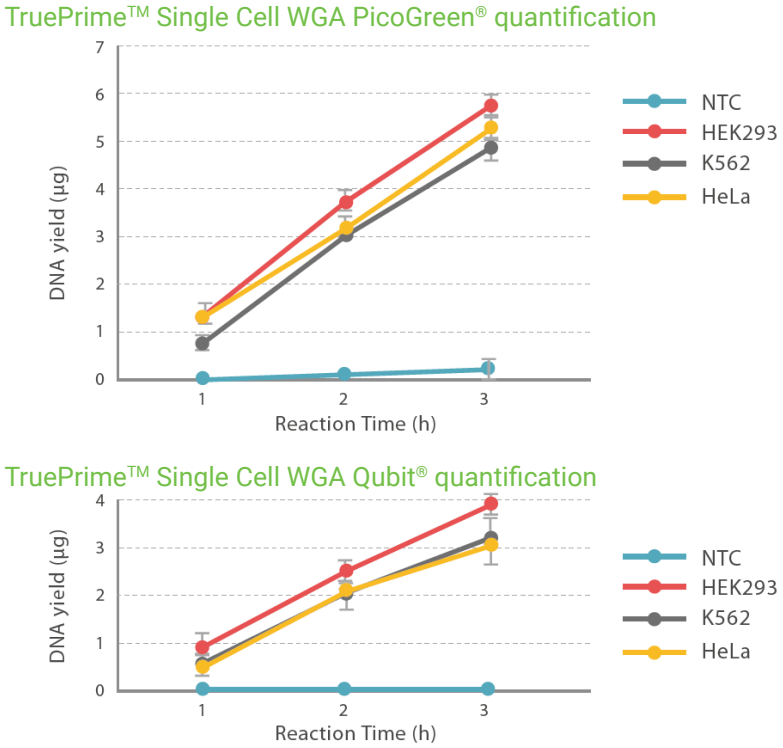

Figure 4. Reaction kinetics of TruePrime™ Single Cell WGA of 3 different human cell lines (NTC = no template control).

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 around 25 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 single cells may vary, leading to different degrees of coverage breadth in cells 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 a PCR panel reflecting coverage of all chromosomes (check for availability from SYGNIS).

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 cell lysis and DNA denaturation** Avoid incubation periods longer than 5 minutes at room temperature or 1 minute at 95°C because it may nick the DNA template and decrease the amplification efficiency. **Cells were not lysed** Additional cell envelope break-down may be necessary for cells that have resistant cell walls.
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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