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# DOPlify®

## Whole Genome Amplification

# TECHNICAL DATA SHEET

Version 1.4

40-46 West Thebarton Road
Thebarton, South Australia, 5031

**DOPlify®** Technical Data Sheet Version 1.4 April 2018



**DOPlify™** is a Research Use Only product and is not to be used for diagnostic procedures

**DOPlify®** is for Research Use Only and should not be used in diagnostic procedures. You are responsible for ensuring that you accurately follow the protocols provided in this Technical Data Sheet (TDS) and analysing and interpreting the results you obtain. PKI(A)does not guarantee any results obtained.

## Contents

# 1 Kit Contents

**Whole Genome Amplification (WGA) – frozen at -20 °C**

| Kit Codes | Component | Cap colour |
|---|---|---|
| RHS4050<br>50 reactions | PCR-grade $H_2O$ | White |
| | Cell Lysis Enzyme | Yellow |
| | Cell Lysis Buffer | Yellow |
| | WGA Polymerase | Red |
| | WGA PCR Buffer | Red |
| | Primer | Red |

# 2 Storage Information

**DOPlify®** kits are shipped on ice packs and remain stable even if the ice packs have thawed and the reagents reach room temperature. Upon receipt, store the kit at -20°C in a constant temperature freezer (not frost free).

Please note that reagents stored at temperatures above -20°C are more prone to degradation and contamination; therefore storage at other temperatures is not recommended. It is not recommended to store reagents at -80°C. When stored under the correct conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label.

This kit is designed to amplify picogram quantities of DNA; therefore extreme caution must be exercised to prevent the introduction of foreign DNA contaminants. Reagents should be stored in a clean laboratory away from potential PCR contaminants. Good laboratory practices for performing PCR should be followed when storing and handling reagents, as well as during the setup of reactions.

# 3 Overview of workflow

**DOPlify® WGA**

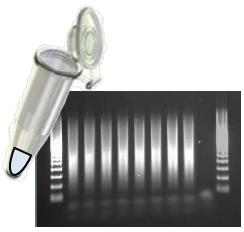
Degenerate Oligonucleotide Primed PCR (DOP-PCR)-based Whole Genome Amplification (WGA) generates representative amplification of total DNA from small numbers of cells or their DNA equivalent. The PKI (A) DOP-PCR-based WGA reproducibly amplifies total DNA from single cells to produce microgram quantities of amplified DNA in less than 3 hours. The kit can be used successfully on both cellular and purified genomic DNA inputs.

*Sample collection*

- Place the sample into a PCR tube in <2 µl of buffer
- Mark the sample location on the tube

*Lysis*

- Add 3 µl of lysis solution above the sample
- Tap the PCR tube to allow the lysis solution to roll over the sample
- Incubate for 15 min according to the Lysis Program



*Whole Genome Amplification (WGA)*

- Add 22 µl of PCR master mix to the lysed sample
- PCR amplify for 2.5 hours using the WGA PCR Program
- Assess WGA quality by agarose gel electrophoresis

# 4 Space, Equipment and Materials to be Supplied by User

**NOTE**: When working with chemicals, always wear a suitable lab coat and disposable gloves. For more information, please consult the appropriate safety data sheets (SDS), available from RHS upon request or reagent supplier.

## 4.1 Laboratory Set Up

**Clean Laboratory**

Cell lysis and master mix set-up for Whole Genome Amplification (WGA) should be performed in a contained and dedicated clean laboratory equipped with a laminar flow hood, dedicated pipettes and a PCR thermocycler with a programmable ramp rate.

It is imperative to maintain a clean, tidy work space with regular decontamination, limiting possible opportunities for DNA contamination.

All DOPlify® WGA reagents should be stored in a -20°C freezer located in the clean laboratory.

**General Laboratory**

Agarose gel electrophoresis and handling of amplified DNA should be performed away from any master mix set-up.

## 4.2 Other Laboratory Items

The cell lysis and WGA steps are recommended to be performed in 0.2 or 0.5mL PCR tubes (supplied by user). A cold block at a temperature of approximately 4°C should be used when preparing all master mixes and for storing samples during set up.

## 4.3 Input Specifications

**Cell Types**
Single blastomeres, polar bodies, trophoblastic cells, amniocytes, lymphocytes, as well as cultured cells, are suitable for amplification using the DOPlify® kit.

**Number of Cells**
The DOPlify® kit is suitable for single cells or its DNA equivalent, as well as small numbers of cells (e.g. <10 cells).

**Cell Collection Method**
Flow sorting, dilution and micromanipulation are collection methods that are compatible with the DOPlify® kit. Single cells should be transferred to a PCR tube with minimal transfer buffer (<2 µl). The location of the cell in the tube should be marked with a dot on the outside of the tube using a permanent marker pen so as to enable easy cell location for the lysis step.

**DNA Dilution**
It is recommended that DNA is diluted to a final concentration of 30pg/µl in 10mM Tris-HCl (pH 8.0) (no EDTA).

**Compatible Buffers**
Recommended cell transfer buffers include 10 mM Tris-HCl (pH 8.0) (no EDTA) and PBS ($Mg^{2+}$, $Ca^{2+}$ free and BSA free).

# 5 Protocols: PKI (A) DOP-PCR Whole Genome Amplification

## 5.1 Cell Lysis

Estimated Time for 4 samples + 1 No Template Control (NTC)

- Hands on: 10 minutes
- Thermocycler: 15 minutes
- Total duration: 25 minutes

In this step, cells are lysed and DNA is made soluble with the addition of a Cell Lysis Mix and a short incubation in a PCR thermocycler.

Consumables:

- 0.2mL or 0.5mL sterile PCR tubes
- PCR-grade $H_2O$
- Cell Lysis Enzyme
- Cell Lysis Buffer
- Cell or DNA samples

Preparation:

- Remove PCR-grade $H_2O$ and Cell Lysis Buffer from the freezer and thaw to room temperature.
- Remove Cell Lysis Enzyme from storage and store in a cold block at 4°C.
- Mix reagents well then briefly centrifuge to collect contents at the bottom of the tube.
- Calculate volumes of reagents required for the Cell Lysis Mix. Enough Cell Lysis Mix should be prepared for all samples and one NTC plus 1-2 reactions extra to allow for pipetting loss. If you are preparing a small number of samples, it is recommended that a minimum of 6-7 reactions are prepared per batch to minimise error from small pipetting volumes.

Procedure:

**NOTE**: The following steps are to be performed in a **Clean Laboratory**.

1. Prepare Cell Lysis Enzyme Dilution 1 in a 4°C cold block by combining the following reagents:

| Component | Volume | Cap Colour |
|---|---|---|
| PCR-grade $H_2O$ | 6.5 µl | White |
| Cell Lysis Enzyme | 1.0 µl | Yellow |
| **Total volume** | **7.5 µl** | |

2. Mix well then briefly centrifuge.
3. Prepare Cell Lysis Mix in a 4°C cold block for the required number of reactions by combining the following reagents:

| Component | Volume for 1 lysis reaction | Recommended minimum volume (6.7x master mix) | Cap colour |
|---|---|---|---|
| PCR-grade $H_2O$ | 2.7 µl | 18 µl | White |
| Cell Lysis Buffer | 0.15 µl | 1.0 µl | Yellow |
| Cell Lysis Enzyme Dilution 1 | 0.15 µl | 1.0 µl | - |
| **Total volume** | **3.0µl** | **20.0 µl** | |

**Steps for Lysing Single Cells/Multi Cell samples**

a. Add 3 µl of Cell Lysis Mix above the cell sample located in a PCR tube. Make sure that the lysis mix rolls over the sample location as marked on the tube by gently tapping the tube on the benchtop. **Do not mix or vortex.**

b. Briefly spin in a mini centrifuge if required to collect contents at the bottom of the tube.

c. Repeat with other samples.

**Steps for NTC Preparation**

a. Add 3 µl of Cell Lysis Mix to 1 sterile PCR tube labelled NTC.

b. Add 1 µl of PCR-grade $H_2O$ to the tube labelled NTC.

**Recommended:** Lyse cell samples and NTC in a **Clean Laboratory** thermocycler to avoid DNA contamination (proceed to Step 4).

**Steps for DNA Sample Preparation prior to WGA PCR (if required)**

a. Add 3 µl of Cell Lysis Mix to the required number of sterile empty PCR tubes.

**NOTE**: The following steps are to be performed in a **General Laboratory**.

b. Add 1µl of 30pg/µl DNA sample to each tube containing Cell Lysis Mix.

c. Proceed to Step 4.

4. Incubate all samples and NTC in a thermocycler programmed as follows:

PKI (A) Lysis

| Process | Temperature | Duration | Cycles |
|---|---|---|---|
| Lysis | 75°C | 10 min | 1 |
| Heat inactivation | 95°C | 5 min | |
| | 4°C | Hold | |

5. Place the lysed samples in a cold block.

## 5.2 Whole Genome Amplification

Estimated Time for 4 samples + 1 NTC

- Hands on: 10 minutes
- Thermocycler: 2 hours 20 minutes
- Total duration: 2 hours 30 minutes

In this step a master mix is created and added to the lysed samples before Degenerate Oligonucleotide Primer PCR (DOP-PCR) based Whole Genome Amplification (WGA). This generates representative amplification of total DNA from cells or their DNA equivalent.

Consumables

- 1.5mL tube
- PCR-grade $H_2O$
- WGA PCR Buffer
- Primer
- WGA Polymerase
- Lysed samples and NTC (from 5.1 Cell Lysis)

Preparation:

- Remove PCR-grade $H_2O$, WGA PCR Buffer and Primer from the freezer and thaw to room temperature.
- Remove WGA Polymerase from storage and store in a cold block at 4°C.
- Mix reagents well then briefly centrifuge to collect contents at the bottom of the tube.
- Calculate volumes of reagents required for the WGA master mix. Enough WGA master mix should be prepared for all samples and 1 NTC plus 1-2 reactions extra.

Procedure:

**NOTE**: The following steps are to be performed in a **Clean Laboratory**.

1. Prepare WGA master mix for the required number of reactions by combining the following reagents in a 1.5mL tube in the order they are listed below:

| Component | Volume for 1 WGA reaction | Cap colour |
|---|---|---|
| PCR-grade $H_2O$ | 6.5 µl | White |
| WGA PCR Buffer | 12.5 µl | Red |
| Primer | 2.5 µl | Red |
| WGA Polymerase | 0.5 µl | Red |
| **Total volume** | **22 µl** | |

2. Mix very well then briefly spin down in a mini centrifuge.
3. Transfer 22 µl of PCR master mix to the individual tubes containing lysed template (sample or NTC in Cell Lysis Mix). To prevent removal of any DNA from the sample, do not insert the pipette tip into lysed sample mix. **Do not mix or vortex the PCR tubes.** Briefly centrifuge or spin in mini centrifuge to collect contents at the bottom of the tube. Proceed to step 5.

**Steps for DNA Sample Preparation prior to WGA PCR (if required)**

**NOTE**: The following steps are to be performed in a **General Laboratory**.

4. Transfer 22 µl of PCR master mix to the individual tubes containing DNA template in Cell Lysis Mix. To prevent removal of any DNA from the sample, do not insert the pipette tip into lysed sample. Mix well then briefly centrifuge to collect contents at the bottom of the tube.
5. Incubate samples and NTC in a thermocycler programmed as follows:

PKI (A) WGA:

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Initial denaturation | 95°C | 5 min | 1 |
| Denaturation | 98°C | 20 sec | 8 |
| Annealing | 25°C | 1 min 30 sec | |
| Extension | Ramp to 72°C | 1°C/4 sec | |
| | 72°C | 1 min | |
| Denaturation | 98°C | 20 sec | 21 |
| Annealing | 58°C | 1 min | |
| Extension | 72°C | 1 min | |
| Final extension | 72°C | 1 min | 1 |
| Cooling | 15°C | Hold | |

## 5.3 WGA Quality Control

To confirm amplification of the DNA sample and to check for contamination in the NTC, it is highly recommended that an agarose gel is run.

Consumables:

- Gel Loading Buffer
- DNA Ladder 100-3000+bp (e.g. Geneworks DMW-100M)
- Agarose
- 0.5x TBE
- WGA products
- Gel Red (Biotium 41003)

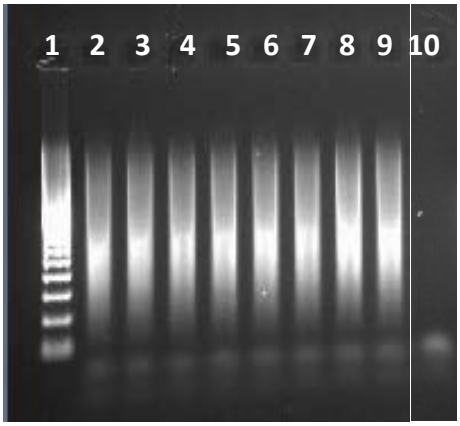
Preparation:

- Create and pour a 1% agarose gel by combining 1g agarose, 100mL 0.5x TBE and 5µL Gel Red. Adjust the volumes to suit the size of your electrophoresis system.

Procedure:

1. Load 2 µl of PCR product in gel loading buffer into the gel.
2. Load DNA Ladder.
3. Electrophorese for ~30 min at 100 volts.

The WGA DNA products should appear as a smear, ranging in size from 200 bp - 2000 bp (see WGA Example 1). The NTC should appear clean, with the presence of primer dimers.




**WGA Example 1:**

Lane 1: DNA Ladder (100bp)

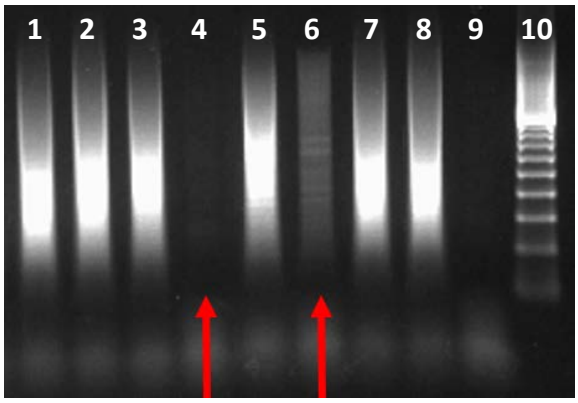
Lanes 2 - 5: Amplified single cells

Lanes 6 - 9: Amplified 30 pg genomic DNA

Lane 10: PCR no template control (NTC)

A **failed** WGA amplification is indicated by the presence of primer dimers, but no evidence of the larger amplification products (see WGA Example 2, lane 4). Possible causes are that the sample was not successfully transferred to the PCR tube or that the sample was located in the PCR tube above the lysis and PCR reagents. Failed samples should be discarded.

**Poor** WGA amplification is indicated by smears with lower intensity or with PCR products that are notably larger or smaller than the expected size range observed for the other samples on the same agarose gel (see WGA Example 2, lane 6). The results from these samples should be interpreted with caution and it is recommended that these samples are discarded.




**WGA Example 2:**

Lanes 1, 2, 3, 5, 7, 8: Amplified single cells

Lane 4: Failed WGA reaction

Lane 6: Poor WGA reaction

Lane 9: NTC

Lane 10: DNA Ladder (100bp)

## 6 Troubleshooting and Technical Support

| Problem | Potential cause | Suggested Solution |
|---|---|---|
| No amplification | Sample was not present in tube | Ensure that method of cell collection consistently results in the sample being accurately transferred to a tube. Position the sample at the bottom of the tube and mark the location with a permanent marker. |
| Low amplification yield | Poor template quality | The DNA in the sample was degraded. This could be due to incorrect storage.<br>Could also be caused by the sample being placed too high in the tube so that the lysis buffer did not cover the sample and it was not completely lysed. Ensure that the sample is placed at the bottom of the PCR tube when collected and that the sample location is marked on the tube. |
| No template control (NTC) generates PCR product evident on the agarose gel | PCR-grade $H_2O$ used to seed the NTC is contaminated with DNA | Replace PCR-grade $H_2O$. |
| | Work area is contaminated with DNA | Clean work area thoroughly and use dedicated PCR pipettes and tips. |
| | Kit reagents have been contaminated | Discard unused reagents and open a new kit. |

For technical support please contact: support@rhsc.com.au

# 7 Ordering Information

Please contact your local distributor or PKI (A) for ordering information. For more information on the products use, limitations, and licenses: www.perkinelmer.com

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PerkinElmer Health Sciences (Australia) Pty Ltd (ABN 84 010 126 708)
40-46 West Thebarton Road THEBARTON SA AUSTRALIA 5031
E: info@rhsc.com.au Ph: +61 88152 9383