DOPlify® v2 Whole Genome Amplification Kit

KIT CONTAINS: 50 RXNS

#4321-0020

DOPlify® v2 Whole Genome Amplification Kit

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You are responsible for ensuring that you accurately follow the protocols provided in this Technical Data Sheet (TDS) and analyzing and interpreting the results you obtain. Revvity does not guarantee any results obtained.

GENERAL INFORMATION

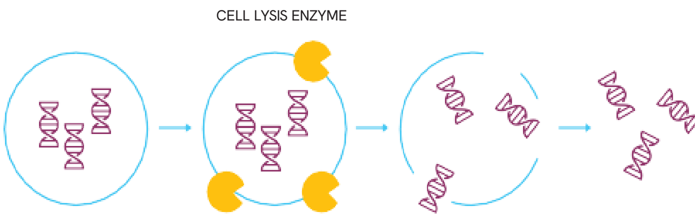
Product Overview

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 kit employs the high processivity and fidelity of new generation, sequencing-grade polymerases, ensuring single base accuracy (1 error in 3.6 x 10^6 bases).

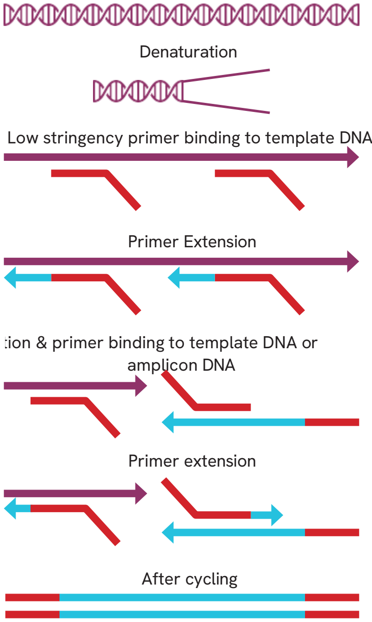
The DOPlify® v2 WGA kit reproducibly amplifies total DNA from limited template samples to produce microgram quantities of amplified DNA in less than 3 hours. The kit can be used successfully on both cellular and purified genomic DNA inputs for a range of different applications including but not limited to massively parallel sequencing (MPS), genotyping and array-based technologies.

The kit follows a single tube workflow, utilizing ready mixes to minimize the pipetting steps needed and requires only two sample tube openings, reducing the risk of sample contamination. In the first step, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for WGA. In the second step, a degenerate primer anneals at regular intervals across the whole genome, assisted by low stringency annealing conditions, and primer extension generates an array of fragments ready for amplification. In the third step, the generated fragments are PCR amplified using high stringency annealing conditions to increase the yield available for down-stream applications.

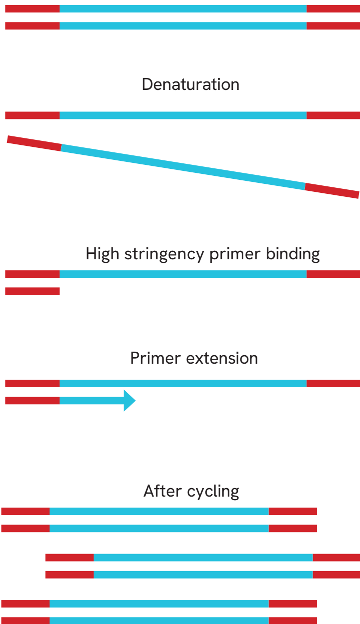
STEP 1: CELL LYSIS



STEP 2: LOW STRINGENCY PCR CYCLES



STEP 3: HIGH STRINGENCY PCR CYCLES



Kit Overview

The DOPlify® v2 WGA Kit includes all reagents necessary for cell lysis and whole genome amplification.

Kit Contents & Storage

Kit Contents	Cap Color	Storage Temp.
DOPlify® v2 WGA Kit (4321-0020) – 50 Reactions		
Cell Lysis Mix	GREEN CAP	-20°C
WGA Ready-mix	ORANGE CAP	-20°C
WGA Primer	BLUE CAP	-20°C

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.

Required Materials Not Provided

- Laminar flow cabinet
- Microcentrifuge
- Pipettes (2, 10, 20, 100, 200, 1000 μL)
- Pipette tips (low binding, barrier filter)
- Thermocycler (with hotlid & programmable ramp rate to 0.25 °C/sec)
- PCR thin walled reaction tube with flat cap (0.5 mL or 0.2 mL)
- Molecular grade tubes (1.5 mL)
- Cold block

Optional Materials Not Provided

- LabChip® GXII Touch™ Nucleic Acid Analyzer and associated reagent kit or Qubit® Fluorometer and associated reagent kit (recommended: Qubit™ 1X dsDNA HS Assay Kit)
- Agarose gel-electrophoresis apparatus
- Electrophoresis power supply
- UV transilluminator or gel documentation instrument

Revision History

Version	Date	Description of Change
v1.0	September 2022	Initial release DOPlify® v2 WGA kit
V2.0	October 2023	Rebrand to Revvity

Warnings & Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.

- Do not use the kit past the expiration date.
- The DOPlify® v2 WGA kit is designed to amplify picogram quantities of DNA; therefore, extreme caution must be exercised to prevent the introduction of foreign DNA contaminants.
- 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® v2 WGA Kit reagents should be stored in a -20°C freezer located in a clean (Pre-PCR) laboratory.
- Handling of amplified DNA should be performed in a general (Post-PCR) laboratory, away from any master mix set-up.
- The cell lysis and WGA steps are recommended to be performed in 0.2 or 0.5 mL PCR tubes (supplied by user).

SAMPLE PREP SETUP

Examples of Suitable Starting Materials

Cells

Limited numbers (1-10) cells, which may include blastomeres, polar bodies, trophoblastic cells, amniocytes, lymphocytes, fibroblast as well as cultured cells, are suitable for amplification using the DOPlify® v2 WGA kit.

Flow sorting, dilution and micromanipulation are collection methods that are compatible with the DOPlify® v2 WGA kit. Limited numbers (1-10) of cells should be washed with PBS (Mg2+, Ca2+ free and BSA free) then 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.

Recommended cell transfer buffers include 10 mM Tris-HCl (pH 8.0) (no EDTA) and PBS (Mg2+, Ca2+ free and BSA free).

It should be noted that processes prior to cell collection that involve staining and/or fixation or other chemical or physical methods may negatively impact the performance of the DOPlify® v2 WGA kit. Please contact https://www.revvity.com/contact-us/technical-support for advice if required.

Genomic DNA

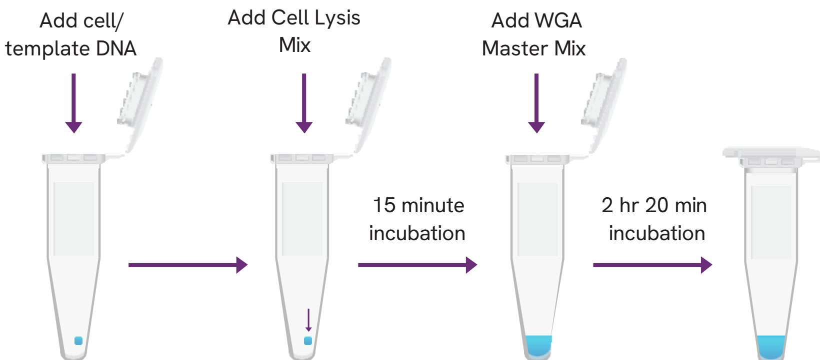
Small amounts of genomic DNA can be used as starting material for WGA. It is recommended that DNA is freshly diluted to a final concentration of 30 pg/µL in 10mM Tris-HCl (pH 8.0) (no EDTA). Genomic DNA dilutions and protocol steps utilising genomic DNA should be performed in a General (Post-PCR) laboratory to prevent contamination.

Reagent Preparation

A cold block at a temperature of approximately 4°C should be used when preparing all master mixes and for storing samples during Cell Lysis and WGA set up.

- Mix well and briefly centrifuge all reagents before use unless specified otherwise.
- Do not vortex the Cell Lysis Enzyme or WGA Polymerase.

Overview of workflow



1.1 Cell Lysis

MATERIALS

- GREEN CAP - Cell Lysis Mix

User Supplied

- Cell or DNA samples
- Thermocycler
- Minicentrifuge
- Cold Block
- 0.2mL or 0.5mL PCR Tubes

! NOTE: The following steps are to be performed in a Clean (Pre-PCR) Laboratory.

1. Prepare samples as described below:

Cell Sample Preparation:

- Add 5 µL of Cell Lysis Mix above the cell sample located in a PCR tube. Make sure that the Cell Lysis Mix rolls over the sample location as marked on the tube by gently tapping the tube on the benchtop.

! NOTE: Do not touch the cell location with the pipette tip. Do not mix or vortex.

- Briefly centrifuge if required to collect contents at the bottom of the tube.
- Repeat with other samples.

Genomic DNA / Positive Control Preparation:

! NOTE: Steps involving genomic DNA are to be performed in a General (Post-PCR) Laboratory.

- Add 5 µL of Cell Lysis Mix to the required number of sterile empty PCR tubes.
- Add 1 µL of 30 pg/µL DNA sample to each tube containing Cell Lysis Mix. DNA should be freshly diluted to 30 pg/µL.
- Briefly Centrifuge.

NTC Preparation (recommended):

- Add 5 µL of Cell Lysis Mix to one sterile PCR tube labelled NTC.
- Add 1 µL of PCR-grade H2O to the tube labelled NTC.
- Briefly centrifuge.

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

10 min	56°C	*1 cycle*
5 min	95°C	*Cycling time is approximately 15 minutes*
Hold	4°C	

3. Place the lysed samples in a cold block.
4. Proceed immediately with Whole Genome Amplification.

1.2 Whole Genome Amplification

MATERIALS

- ORANGE CAP - WGA Ready-mix
  (Thaw to room temperature. Mix well then briefly centrifuge)
- BLUE CAP - WGA Primer
  (Place in a cold block. Mix well then briefly centrifuge.)

User Supplied

- Lysed samples and NTC (from Step 1.1)
- Thermocycler
- Minicentrifuge
- Cold block
- Molecular grade tube

! NOTE: The following steps are to be performed in a Clean (Pre-PCR) Laboratory.

1. Calculate the volumes of reagents required to prepare the WGA PCR Master Mix for all samples, one NTC plus 10% extra volume to allow for loss during pipetting.
2. Prepare WGA PCR master mix for the required number of reactions by combining the following reagents in the order they are listed below:

Volume for 1x WGA PCR Reaction	Component
20 µL	WGA PCR Ready-mix
15 µL	WGA Primer
35 µL	TOTAL

3. Mix very well then briefly centrifuge.
4. Transfer 35 µL of WGA PCR master mix to the individual tubes containing lysed template (sample or NTC in Cell Lysis Mix).

! NOTE: To prevent removal of any DNA from the sample, do not insert the pipette tip into lysed sample mix.

! NOTE: Do not mix or vortex the PCR tubes.

5. Briefly centrifuge.

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

Initial denaturation	5 min	95°C	*1 cycle*
Denaturation	20 sec	98°C	*8 cycles*
Annealing	1 min 30 sec	25°C	
Extension	0.25°C/sec	Ramp to 72°C	
	1 min	72°C	
Denaturation	20 sec	98°C	*15-21 cycles**
Annealing	1 min	58°C	
Extension	1 min	72°C	
Final extension	1 min	72°C	*1 cycle*
Cooling	Hold	4°C	

**The number of cycles can be modified according to the starting template. Cycling time is approximately 2 hours – 2 hours and 20 minutes, depending on the number of cycles chosen*

! NOTE: The slow ramping speed in the first 8 PCR cycles are the low stringency conditions required for the DOPlify® kit Whole Genome Amplification. It is critical that this condition is achieved. If you are unsure about the suitability of your thermocycler, please contact https://www.revvity.com/contact-us/technical-support for advice.

STOPPING POINT:
If you are stopping sample preparation here, store WGA PCR Products at -25 °C to -15 °C.

7. (Optional QC) It is recommended that users run each sample on the Revvity LabChip® GXII Touch™ instrument to observe sample quality and yield. Alternatively, users can analyse samples using agarose gel electrophoresis and Qubit® dsDNA Assay (Life Technologies) quantification. See Appendix A for quality control information.

! NOTE: If the quality of the WGA was not as expected, see Appendix B for troubleshooting information.

! NOTE: For downstream applications, it is recommended that the WGA PCR Product is purified using a 1x SPRI bead purification before use.

APPENDIX A

Electrophoresis

The DOPlify® v2 WGA Kit DNA products should appear as a smear, ranging in size from approximately 200 bp - 3000 bp. The NTC should appear clean, with the presence of primer dimers (Agarose Gel Electropherogram: Figure 1, LabChip® Electropherogram: Figure 2).

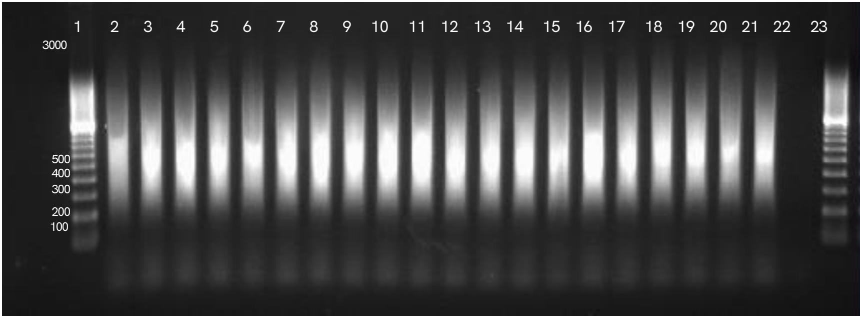


Figure 1: Agarose gel electropherogram of WGA DNA products of 5-cell aliquots from a fibroblast cell culture. Samples were run on a 0.5x TBE 1% agarose gel at 100 V for 30 minutes. Lanes 1 and 23: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-21: WGA DNA products, Lane 22: No Template Control.

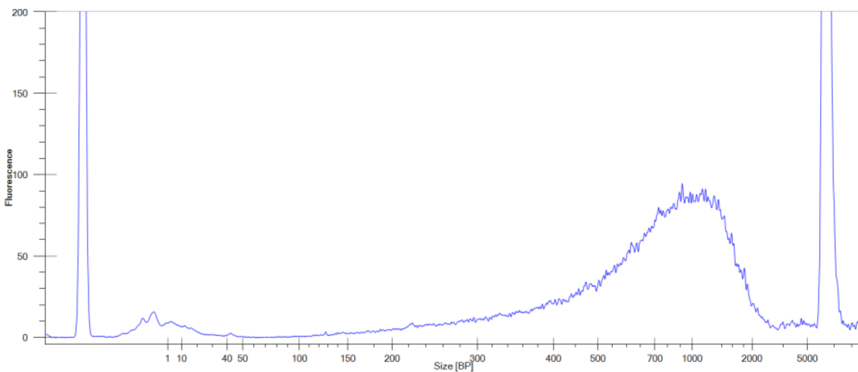


Figure 2: LabChip® electropherogram of a DOPlify® kit whole genome amplified sample which showed expected amplification. The sample was diluted to 1 ng/µL before being analysed using the LabChip DNA High Sensitivity Reagent Kit.

APPENDIX B

Troubleshooting

Problem	Potential Cause	Suggested Solution
No amplification	Sample was not present in the sample collection tube. Sample was located too high in the PCR tube, above the Cell Lysis and PCR reagents	Ensure that the 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.

A failed WGA amplification is indicated by the presence of primer dimers, but no evidence of the smear of amplification products (Agarose Gel Electropherogram: Figure 3, LabChip® Electropherogram: Figure 4). Failed samples should be discarded.

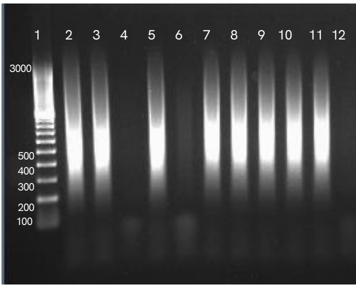


Figure 3: Agarose gel electropherogram of DOPlify® v2 kit WGA DNA products. Samples were run on a 0.5x TBE 1% agarose gel at 100 V for 30 minutes. Lanes 1: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-11: WGA DNA products, Lane 12: No Template Control. Lanes 4 and 6 showed a failed WGA reaction.

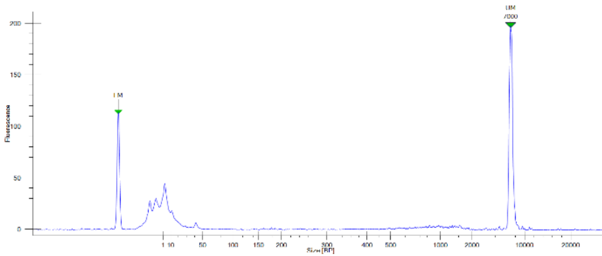


Figure 4: LabChip® electropherogram of a sample which failed WGA. The sample was diluted to 1 ng/µL before being analysed using the LabChip® DNA High Sensitivity Reagent Kit.

Problem	Potential Cause	Suggested Solution
Low amplification yield	Poor template quality	The DNA in the sample was degraded. This could be caused by incorrect storage, PCR inhibitors present in the sample, or the sample being placed too high in the tube causing incomplete lysis. Ensure that samples are of high quality and confirm PCR inhibitors such as EDTA, proteins, detergents or salts are

		not present in the sample. Make sure that the sample is placed at the bottom of the PCR tube when collected and that the sample location is marked on the tube.

Poor WGA amplification is indicated by smears with weaker yields or PCR products that are notably larger or smaller than the expected size range observed for the other samples on the same agarose gel (Agarose Gel Electropherogram: Figure 5, LabChip Electropherogram: Figure 6). It is recommended that these samples are removed from further analysis or that the results from these samples are interpreted with caution.

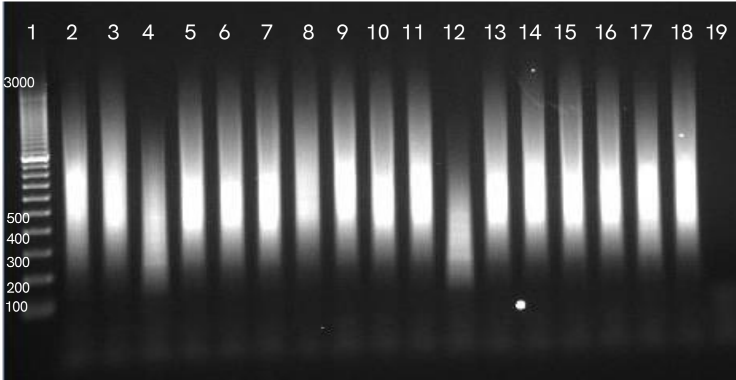


Figure 5: Agarose gel electropherogram of DOPlify® v2 WGA Kit DNA products. Samples were run on a 0.5x TBE gel at 100 V for 30 minutes. Lanes 1: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-18: WGA DNA products, Lane 19: No Template Control. Lanes 4 and 12 show a weak WGA reaction with smaller then expected amplicon fragment size.

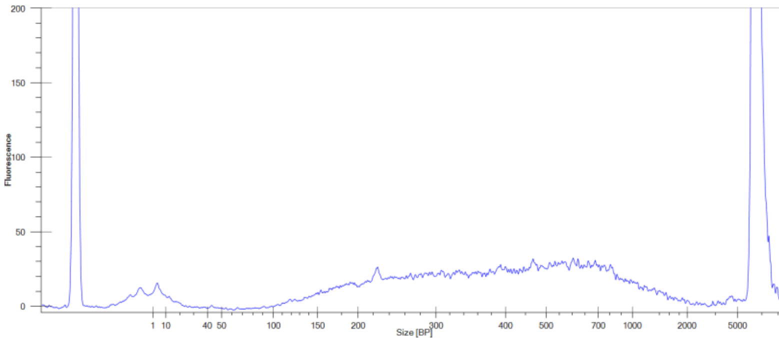


Figure 6: LabChip® electropherogram of a sample which showed weak amplification. This sample presented a smaller than expected smear with reduced intensity. The sample was diluted to 1 ng/µL before being analyzed using the LabChip® DNA High Sensitivity Reagent Kit.

Problem	Potential Cause	Suggested Solution
No template control (NTC) generates PCR products evident on the agarose gel	PCR-grade H2O used to seed the NTC is contaminated with DNA	Replace PCR-grade H2O
	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

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