Product Profile

REPLI-g® Advanced DNA Single Cell Kits

Improve your workflow and data consistency – whole genome amplification from single eukaryotic cells, limited sample amounts or isolated genomic DNA

The REPLI-g® Advanced DNA Single Cell Kit is an enhanced single cell DNA amplification kit that uses the Phi 29 polymerase in a Multiple Displacement Amplification (MDA) reaction. This single cell kit features advanced cell lysis and improved chemistry that result in a more robust reaction with higher uniformity and lower allelic dropout rates. The use of a novel single cell cryo-protective reagent (included with the kits) protects DNA from damage during single cell collection and long term storage.

REPLI-g Advanced DNA Single Cell Kits provide:

- Fewer amplification errors: Phi 29 polymerase for 1000x greater accuracy than Taq-based methods
- More consistent performance: new methodology increases consistency across applications, users and sites
- Improved coverage: new chemistry increases uniformity and reduces amplification bias
- Faster workflow: simple 2 hour and 20 minute protocol from start to finish
- Compatibility with microarray, qPCR and NGS analysis

Setting the standard for sensitive applications

REPLI-g Advanced DNA Single Cell Kits provide superior enzymatic performance consistency, resulting in high-level variant-calling accuracy for single cell and low-input applications. When SNV and CNV are of equal importance (for example, when analyzing aneuploidy and sub-chromosomal copy number variations), high sequence fidelity and contig mapping minimize challenges of molecular analyses. In addition, REPLI-g Advanced DNA Single Cell Kits provide greater yield than other methods, so you have more downstream analysis options (Figure 1, next page).

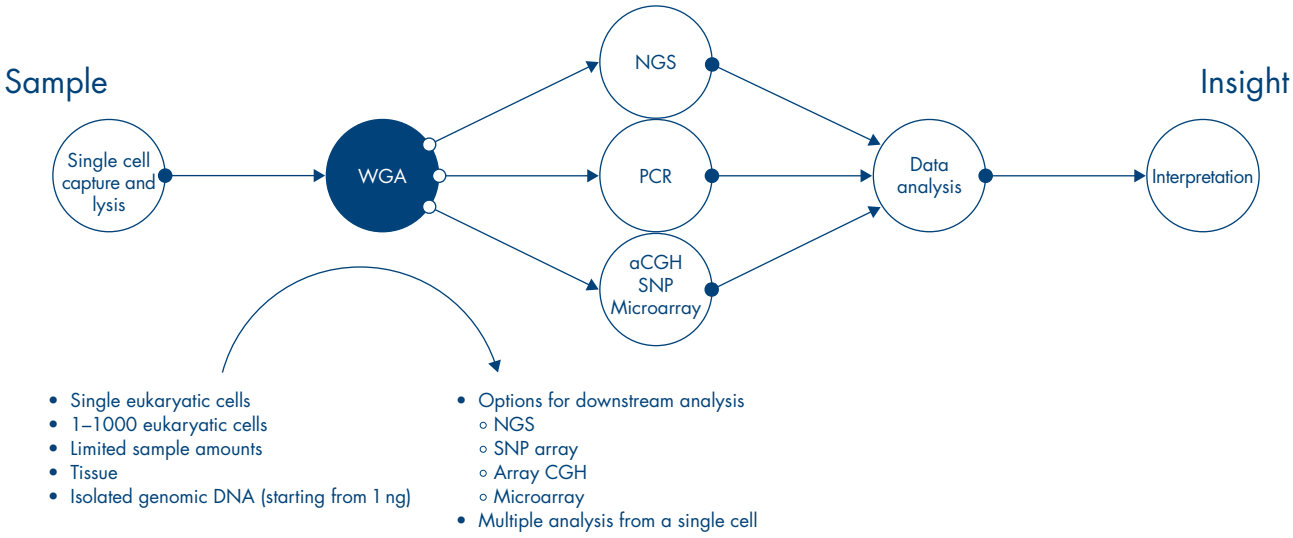

Figure 1. REPLI-g Advanced DNA Single Cell Kits can be used to amplify small amounts of material for downstream genomics applications.

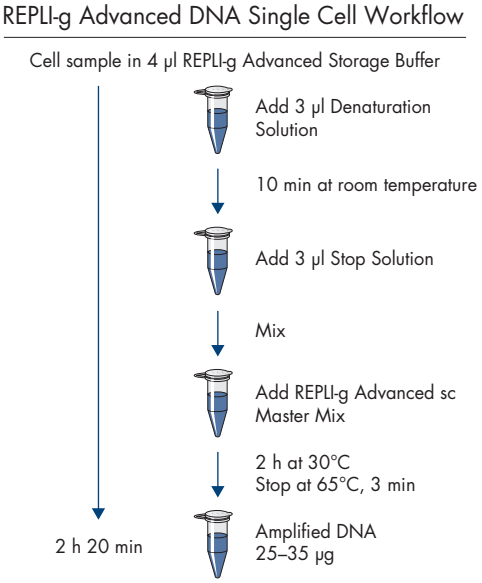

Figure 2. REPLI-g Advanced DNA Single Cell protocol. The REPLI-g Advanced DNA Single Cell Kit features a simplified protocol of just 3 steps with only 20 minutes hands-on time for reaction setup and 2 hours for reaction time. The typical yield from a single cell is 25–35 µg of amplified DNA – sufficient for downstream applications involving qPCR and next-generation sequencing. The REPLI-g Advanced DNA Single Cell protocol saves at least 1 hour of time versus the first-generation REPLI-g Single Cell Kit (cat. nos. 150343 and 150345).

Simple, optimized workflow

The new REPLI-g Advanced DNA Single Cell Kit has a simplified protocol of only 3 steps to go from single cell to more than 25 µg of amplified DNA (Figure 2). The first step involves a room temperature alkaline cell lysis that gently breaks open the cell and makes the DNA accessible for amplification. After stopping the lysis reaction, the MDA reaction takes place for 2 hours, resulting in 25–35 µg of DNA.

REPLI-g Advanced DNA Single Cell Kits were tested in multiple experiments by 2 operators, using 4 different lots of reagents, with 4 different types of eukaryotic cells.

Single cells were isolated with the QIAGEN® QIAscout®. Single cells are estimated to contain 6 pg of DNA which is an insufficient input amount for most genomic downstream applications, such as microarray, qPCR or NGS analysis.

In less than three hours, the REPLI-g Advanced DNA Single Cell Kit makes single cell genomic studies possible and reliable (Table 1, next page).

In our experiments, we compared the single allelic dropout rate of the new REPLI-g Advanced DNA Single Cell Kit with the original, first-generation REPLI-g Single Cell DNA Kit by analyzing heterozygous SNPs. The REPLI-g Advanced DNA Single Cell Kit shows reduced allelic dropout with improved consistency when using targeted DNA sequencing as a downstream assay (Figure 3, next page).

Furthermore, targeted NGS analysis revealed that the rate of allelic dropout is decreased after storage of single cells in REPLI-g Single Cell Cryo-Protect Reagent when compared to single cells stored in single cell PBS. The lower allelic dropout rate demonstrates the protective effect of the REPLI-g Single Cell Cryo-Protect Reagent, which reduces damage of the single cell and its DNA during freezing and storage (Figure 4, next page).

Table 1. REPLI-g Advanced DNA Single Cell Kit provides consistent results

Cell line	Number of single cells analyzed	Experiment number	Average yield (µg)	Standard deviation (µg)	% CV	Reagent lot
Jurkat	34	1	28.8	3.2	11.1%	A
Jurkat	18	2	29.8	2.0	6.7%	A
LoVo	47	1	27.8	2.1	7.6%	A
MCF7	48	1	35.8	2.5	7.0%	B
HeLa	47	1	29.1	2.9	10.0%	B
Jurkat	5	3	39.6	4.0	10.2%	C
Jurkat	13	4	38.5	2.7	7.1%	D

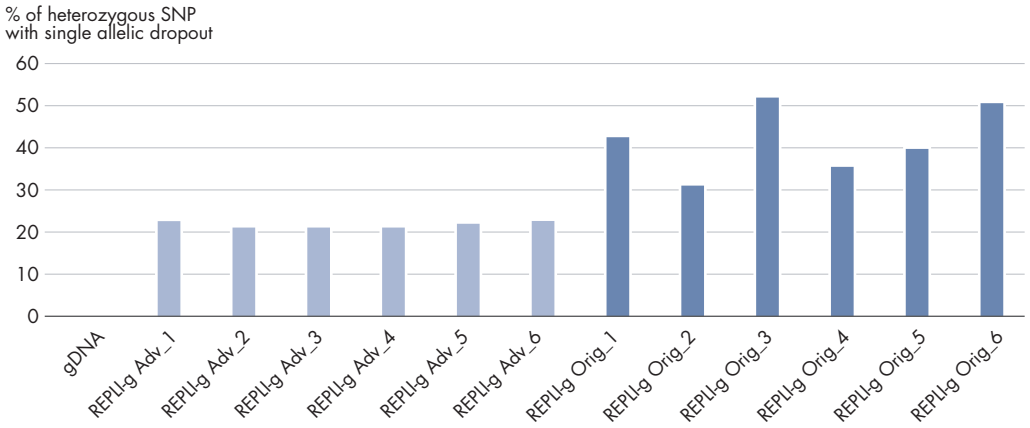

Figure 3. REPLI-g Advanced DNA Single Cell Kit reduced allelic dropout and improved consistency. Single allelic dropout (ADO) for REPLI-g–amplified DNA from single cells was assessed using libraries generated using the QIAseq Targeted DNA Human Comprehensive Cancer Panel and sequenced using a MiSeq® (Illumina®). A comparison between 6 single Jurkat cells using the REPLI-g Advanced DNA Single Cell kit and 6 single Jurkat cells using the original REPLI-g Single Cell Kit is shown. Unamplified gDNA from bulk Jurkat cells was used as reference. NGS results were analyzed using the GeneGlobe Data Analysis Center (**www.qiagen.com/shop/genes-and-pathways/data-analysis-center-overview-page/**).

Each library is based on a single cell after WGA, with 40 ng amplified DNA used as starting material, and a mean read depth of 127. To compare the ADO rates for both kits, we analyzed the single ADO for SNPs that are heterozygous (frequency of SNP allele: 25 – 75 %) in the unamplified bulk DNA. Calculation of ADO rate is: (# of heterozygous loci in gDNA – # of heterozygous loci in single cell after WGA)/(# of heterozygous loci in gDNA).

The REPLI-g Advanced DNA Single Cell Kit shows a lower rate of ADO and improved inter-sample consistency.

In conclusion, the REPLI-g Advanced DNA Single Cell Kit is designed for whole genome amplification from single eukaryotic cells, limited sample amounts or isolated genomic DNA. Using this kit can help improve your workflow and data consistency – increasing your productivity and giving you greater confidence in your results.

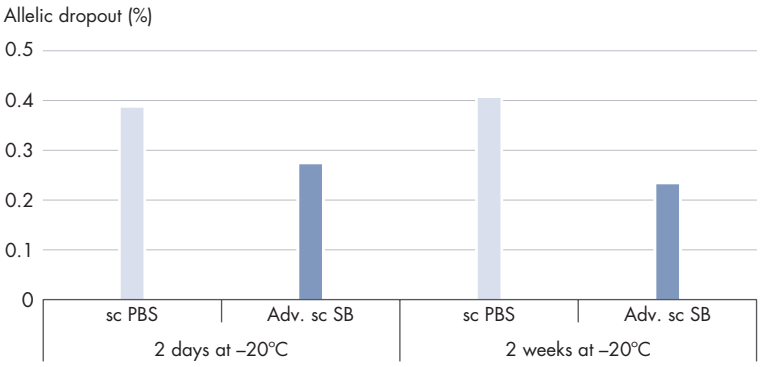

Figure 4. REPLI-g Single Cell Cryo-Protect Reagent provides reduced allelic dropout rates. Single cells were isolated using the QIAscout and either transfered into single cell PBS (sc PBS) or into REPLI-g Single Cell Cryo-Protect Reagent (Adv. sc SB). Cells were stored at –20°C for either 2 days or 2 weeks before WGA was performed. Single cells stored in Adv. sc SB were processed using the REPLI-g Advanced DNA Single Cell Kit and single cells stored in sc PBS were processed using the original REPLI-g Single Cell Kit.

Single allelic dropout (ADO) for REPLI-g–amplified DNA from single cells was assessed using libraries generated using the QIAseq Targeted DNA Human Comprehensive Cancer Panel and sequenced using a NextSeq® (Illumina). For each condition, the mean of 4 libraries is shown. Each library is based on a single cell after WGA, with 40 ng amplified DNA used as starting material. Analysis of ADO is calculated by heterozygous SNPs as described for Figure 3.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Advanced DNA Single Cell Kit (24)	REPLI-g sc Polymerase, buffers, and reagents for 24 x 50 µl whole genome amplification reactions (typical yield 30 µg per reaction)	150363
REPLI-g Advanced DNA Single Cell Kit (96)	REPLI-g sc Polymerase, buffers, and reagents for 96 x 50 µl whole genome amplification reactions (typical yield 30 µg per reaction)	150365
REPLI-g Single Cell Cryo-Protect Reagent (15 ml)	REPLI-g Advanced sc Storage Buffer (8 tubes)	150370

The REPLI-g Advanced DNA Single Cell Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.