PicoPlex™ WGA Kit

For Single-Cell Whole Genome Amplification

Product Number R30050 (50 reactions)

Storage: -20°C

FOR RESEARCH USE ONLY

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A. Kit Components

Component Name (ID)	Volume
Cell Extraction Buffer (R30050-01)	300 µL
Extraction Enzyme Dilution Buffer (R30050-02)	300 µL
Cell Extraction Enzyme (R30050-03)	15 µL
PicoPlex Pre-Amp Buffer (R30050-04)	275 µL
PicoPlex Pre-Amp Enzyme (R30050-05)	15 µL
PicoPlex Amplification Buffer (R30050-06)	1.4 mL
PicoPlex Amplification Enzyme (R30050-07)	50 µL
Nuclease-Free Water (R30050-08)	1.8 mL
User Manual (R30050-09)	

B. Storage and Handling

Store the PicoPlex WGA Kit at -20°C. Transfer Cell Extraction Enzyme, Pre-Amp Enzyme, and Amplification Enzyme tubes to ice just before use. Thaw other components on ice and briefly vortex component tubes prior to use.

Since this kit is designed to amplify picogram quantities of DNA, extreme caution must be exercised to prevent foreign DNA contaminants. Reagents should be stored, handled, and reaction setups performed following good laboratory practices for performing PCR.

C. Additional Required Materials

- Thermal cycler (Real-time instrument recommended)
- PCR tubes or 96-well PCR plate (see Section I)
- PCR plate seals (see Section I)
- Low-binding barrier tips
- PBS Buffer (see Section F)

D. Product Description

The PicoPlex WGA Kit reproducibly amplifies total DNA from single cells about 1 million fold to produce 2-5 micrograms of amplified DNA in under 3 hours.

The PicoPlex WGA Kit delivers the following superior features:

- 100% amplification success rate with flow-sorted cells
- Faithful representation of even GC-rich genomic regions
- Greater than 90 % correlation coefficient for Q-PCR Ct data from replicate single-cell reactions
- Single-copy sensitivity and high specificity, with expected 5 PCR cycle delay between WGA of single cells and WGA of buffer controls.

E. Applications for PicoPlex WGA Products

The PicoPlex WGA Kit produces amplified DNA fragments suitable for:

- Copy number variation (CNV) using oligonucleotide aCGH or QPCR
- SNP genotyping
- Mutation detection
- Sequencing

F. Cell Specifications

Cell Types
Single blastomeres, polar bodies, trophoblastic cells, amniocytes, and cultured cells have been amplified by PicoPlex. For most reproducible results with cultured cells, Rubicon recommends using homogeneous cell lines with stable karyotypes.

Number of Cells
PicoPlex is uniquely suited for single cell amplifications, since the same robust, reproducible amplification is obtained from single cells as from large numbers of cells (e.g., 1,000 cells). Although PicoPlex can be used to amplify multiple cells, the greatest PicoPlex advantage over other WGA technologies will be obtained with single cells.

Cell Collection Methods
Flow sorting, dilution, and micromanipulation are collection methods that are compatible with the PicoPlex WGA Kit. Cell staining may negatively affect kit performance. Formalin fixation must be avoided to achieve optimum results.

Washing Cells
Cell washing is strongly recommended to minimize non-cellular DNA contamination of the cell preparation. Mg^{2+}-free, Ca^{2+}-free, BSA-free PBS may be used for washing, and 1x PBS prepared from the following stock solutions is recommended:

- 10x PBS (USB Corporation, Catalog# 75889)
- 20x PBS (Cell Signaling Technology, Inc., Catalog# 9808)

Wash buffers containing Mg^{2+}, Ca^{2+}, or BSA must be avoided.

The PBS volume carried over with the cell sample into the PicoPlex Protocol cannot exceed 2.5 µL.

G. Using Amplified Control DNA as Reference

Control DNA samples are useful references for some analytical platforms such as microarrays and QPCR. For the most accurate results, Rubicon strongly recommends that PicoPlex-amplified samples are compared to PicoPlex-amplified control DNA rather than un-amplified control DNA.

Control DNA samples must be prepared according to Sample Preparation Methods (Section J) and amplified using the procedure specified in the PicoPlex protocol (Section K). Best results will be obtained by pooling the products of multiple corresponding control DNA amplification reactions.

H. Purifying and Quantifying PicoPlex Products

Many applications require purifying and quantifying WGA products before use. PicoPlex products can be purified with spin columns or filter plates. Rubicon has validated the following purification systems:

- DNA Clean & Concentrator™-5 Kit (Zymo Research, Catalog# D4014)
- MultiScreen PCR_{96} plate (Millipore, Catalog# MSNU03050)

Quantify purified products by UV absorbance (1 OD260 = 50 µg/mL). PicoGreen™ or other double-strand specific measurements will not give reliable PicoPlex product concentrations.

I. Selecting Appropriate Reaction Tubes/Plates

Considerable (>5 µL) evaporation may occur during PicoPlex Protocol Step 6 if the incubation is being performed in a PCR tube or plate without a tight seal, and such evaporation may reduce the robustness and reproducibility of PicoPlex WGA.

A mock PicoPlex Protocol Step 6 incubation using 15 µL of water is advised to confirm whether a selected tube or plate/seal combination can be used with minimal volume loss due to evaporation.

Rubicon has observed minimal evaporation with the following PCR plate and seal combination:

- iCycler IQ 96-well PCR plates (Bio-Rad, Catalog# 223-9441)
- Axymat silicone sealing mats (Axygen, Catalog# AM-96-PCR-RD)

J. Sample Preparation Methods

5 µL Cell Sample

1. **Wash or dilute cells with PBS buffer, according to instructions in Cell Specifications (Section F).**

2. **<u>If collecting cells by flow sorting</u>:**
   Collect a single cell into 5 µL of Cell Extraction Buffer in a PCR tube or well.

   <u>If collecting cells by micromanipulation or dilution</u>:
   Transfer a single cell in minimal PBS volume (<2.5 µL) to a PCR tube or well containing an appropriate volume of Cell Extraction Buffer to achieve a total cell sample volume of 5 uL.

3. **Immediately freeze and store cells at -80°C or proceed directly to the PicoPlex Protocol (Section K).**

5 µL Control DNA Sample (single cell simulation)

1. **Prepare a 1 ng/µL purified DNA solution in a PCR tube or well by diluting a control DNA stock with 5 mM Tris-HCl (pH 8.0).**

2. **Vortex the 1 ng/µL DNA solution for 30 seconds.**

3. **Add 3 µL of the 1 ng/µL DNA solution to 197 µL of 5 mM Tris-HCl (pH 8.0) to prepare a 15 pg/µL DNA solution.**

4. **Vortex the 15 pg/µL DNA solution for 30 seconds.**

5. **Add 1 µL of the 15 pg/µL DNA solution to 4 µL of Cell Extraction Buffer in a PCR tube of well.**

K. PicoPlex Protocol

1. **Combine Extraction Cocktail components and mix well.**

Extraction Cocktail	Volume Per 5 samples
Extraction Enzyme Dilution Buffer	24 µL
Cell Extraction Enzyme	1 µL
Total Volume	**25 µL**

2. **Add 5 µL of freshly-prepared Extraction Cocktail to each 5 µL Cell Sample or Control DNA Sample prepared in Section J.**

3. **Incubate sample in a thermal cycler as follows:**

1 cycle	**75°C**	**10 min**
1 cycle	**95°C**	**4 min**
1 cycle	**Room Temp**	**Hold**

4. **Combine Pre-Amp Cocktail components and mix well.**

Pre-Amp Cocktail	Volume Per 5 samples
PicoPlex Pre-Amp Buffer	24 µL
PicoPlex Pre-Amp Enzyme	1 µL
Total Volume	**25 µL**

5. **Add 5 µL of Pre-Amp Cocktail to each cell or control sample.**

6. **Incubate sample according to thermal cycler program below:**

1 cycle	**95°C**	**2 min**
12 cycles	**95°C**	**15 sec**
	15°C	**50 sec**
	25°C	**40 sec**
	35°C	**30 sec**
	65°C	**40 sec**
	75°C	**40 sec**
1 cycle	**4°C**	**hold**

K. PicoPlex Protocol (continued)

7. **Briefly centrifuge sample and place Pre-Amp incubation product on ice.**

8. **Combine the following Amplification Cocktail components and mix well.**

Amplification Cocktail	Volume Per 5 Samples
PicoPlex Amplification Buffer	125 µL
PicoPlex Amplification Enzyme	4 µL
Nuclease-Free Water	171 µL
Total Volume	**300 µL**

Note: Sample amplification efficiency may be analyzed using a real-time thermal cycler by adding SYBR Green I dye (Invitrogen, Catalog# S7563) at 0.125x final concentration in the Amplification Cocktail (see Appendix A). Some instruments require additional dyes for signal normalization.

9. **Mix 60 µL of the freshly prepared Amplification Cocktail with the 15 µL Pre-Amp incubation product and mix by pipet.**

10. **Amplify sample according to thermal cycler program below:**

1 cycle	95°C	2 min
14 cycles	95°C	15 sec
	65°C	1 min
	75°C	1 min

Note: 14 cycles is <u>recommended</u> based on Rubicon testing performed with flow-sorted cultured cells. Some cell types may require additional cycles (up to 16) to obtain maximal yields.

11. **Immediately store the amplified PicoPlex product at -20°C or purify (see Section H) and store the purified, amplified PicoPlex product at -20°C.**

Appendix A: Analyzing Amplification Efficiency

Sample amplification efficiencies can be analyzed by performing the amplification reactions with SYBR® Green I in a real-time thermal cycler. During the amplification reaction, double-stranded amplified molecules are bound by the non-sequence-dependent SYBR® Green I dye, and the accumulation of amplified product is detected as an increase in fluorescence by the real-time instrument.

Data analysis should be performed on raw background-subtracted (not baseline cycle normalized) fluorescence, and the instrument/software should be set to the appropriate mode.

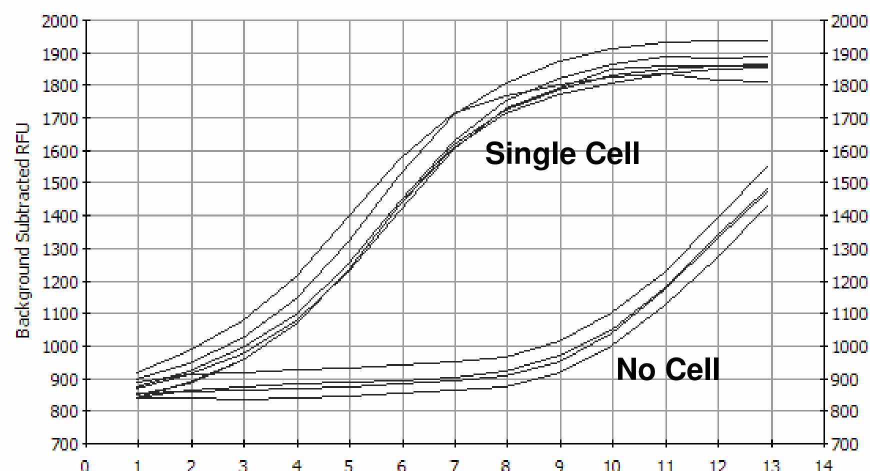

Figure 1: Example of Background Subtracted RFU amplification curves for replicate single-cell and control no-cell WGA reactions that were monitored on a Bio-Rad I-Cycler iQ.

Amplification curves will have a similar appearance for all single-cell PicoPlex WGA reactions, with an immediate 8-9 cycle upward sloping phase, followed by a relatively flat "plateau" phase (Figure 1).

No-cell control amplification curves are delayed (right-shift) by 5-6 PCR cycles compared to single-cell amplification curves. A smaller delay of control curves may indicate DNA contamination introduced with the sample or during the WGA process.

Appendix B: Troubleshooting Guide

Problem	Potential Cause	Suggested Solutions
Single-cell amplification curve looks like control no-cell amplification curve or does not produce amplified product	Sample tube or well did not contain a cell	Confirm that cell collection method reproducibly results in single cell per tube or well
	Improper sample preparation	Follow instructions in Cell Specifications (Section F)
	Improper purification or quantification	Follow instructions for Purifying and Quantifying PicoPlex products (Section H)
Single-cell amplification curve reaches "plateau" phase earlier 15 pg control DNA reaction	Greater than one cell in sample	Confirm that cell collection method reproducibly results in single cell per tube or well
	Single cell sample is contaminated with extraneous DNA	Use fresh, BSA-free PBS
Control no-cell amplification curve appears early or produces yield similar to single-cell reaction products	Control solution is contaminated with DNA	Use fresh control solution
	Work area is contaminated with DNA	Clean area thoroughly and use PCR-dedicated plastics and pipettes.
	Kit has become contaminated with DNA	Use fresh kit