MALBAC® Single Cell WGA Kit Manual

PRODUCT NAME

Universal Name: MALBAC® Single Cell WGA Kit

SIZE / CAT. NO.

Size	Cat. No.
10 rxns	KT110700110
50 rxns	KT110700150

INTENDED USES

The amplified products by the MALBAC Single Cell WGA Kit can be used on various analytical platforms such as real-time PCR (qPCR) and next-generation sequencing. It is also applicable to other downstream experiments and analyses: point mutation detection by Sanger sequencing; SNP genotyping ; CNV profiling and aneuploidy detection; Array CGH; SNP array **.**

BASIC PRINCIPLE

MALBAC Single Cell WGA Kit generates highly uniform amplification across the entire genome. The method is based on the patented technology – MALBAC (Multiple Annealing and Looping Based Amplification Cycles). MALBAC utilizes a mixture of highly processive DNA polymerases with strong strand displacement activity to carry out quasilinear pre-amplification cycles of the genome, followed by exponential amplification (regular PCR) to yield sufficient amount of genomic DNA for various downstream analyses.

COMPONENTS

Component	Cap Color	Voume & Quantity	
		10 rxns	50 rxns
Cell Lysis Buffer	Blue	50 μL * 1	250 μL * 1
Cell Lysis Enzyme	Blue	5 μL * 1	25 μL * 1
Pre-Amp Buffer	Green	300 μL * 1	750 μL * 2
Pre-Amp Enzyme	Green	10 μL * 1	50 μL * 1
Amplification Buffer	Red	300 μL * 1	750 μL * 2
Amp Enzyme	Red	8 μL * 1	40 μL * 1

EQUIPMENT & CONSUMABLES SUPPLIED BY USERS

1. Consumables: Nuclease-free water, 1×PBS Buffer.
2. Equipment: Thermal cycler, Micro spectrophotometer, Vortexer, Microcentrifuge.

STORAGE & PERIOD OF VALIDITY

Store Condition: -25～-15℃; avoid repeated freeze-thaw cycles.
Period of Validity: 24 months; MFD / EXP: On the package.

PRODUCT FEATURES

1. 2 to 5 μg DNA generated from single cell whole genome amplification.
2. tube, 3-step, 4-hr process. No purification required for intermediate products.
3. 95% amplification success rate for single cells sorted by flow cytometry or starting genomic DNA of > 0.5pg.
4. Reproducible locus representation and consistent amplification efficiency in both AT- and GC-rich regions.
5. High coverage with the locus dropout rate less than 10%.

RESEARCH AREAS

MALBAC Single Cell WGA Kit is an innovative tool for whole genome amplification from various starting materials including genomic DNA, cultured cells, sperm, oocyte, semen stains, fresh or dried blood, fresh or frozen tissue and other trace forensic evidence. Applicable research fields include:

1. **Human and applied animal biology:** Biomarker study (CNVs, SNVs); pre-implantation genetic screening and diagnostic (PGS/PGD); genotyping of transgenic animals; embryo, single sperm genotyping, stem cell and neuron research.
2. **Cancer research:** Somatic mutation analysis; tumor heterogeneity, development/evolution; cancer stem cells; circulating tumor cells (CTCs).
3. **Microbiology:** Metagenomics; microbial genotyping.

上海亿康医学检验所有限公司
电话：400-921-3918
官网：www.yikongenomics.com
地址：上海市奉贤区望园路1698弄聚惠领域26号

SAMPLE SPECIFICATION

1. **Sample Amount:** MALBAC Single Cell WGA Kit is specifically designed for single-cell whole genome amplification. The kit is also suitable for samples that is a single chromosome or 0.5 pg to 1 ng of genomic DNA.
2. **Collection Method:** MALBAC Single Cell WGA Kit is compatible with the following collection methods: flow cytometer sorting, dilution by buffer, micromanipulation, laser capture microdissection, buccal cells, biopsy samples. The kit is also suitable for the tissue samples obtained by paraformaldehyde fixation and laser capture microdissection.
3. **Pre-treatment of Samples:** Cell washing is strongly recommended prior to the experiment to avoid the contamination of exogenous DNA during cell preparation. Use Ca^{2+} and Mg^{2+} free 1xPBS solution for washing. Wash buffers containing Mg^{2+}, Ca^{2+} must be avoided. Note that the volume of PBS solution carried over with the cell sample into the amplification should not exceed 2 µL.

PREPARATION BEFORE EXPERIMENT

1. **Isolation of Pre-Amplification Sample Preparation Working Area**

- Prior to amplification, sample preparation should be performed in a designated laboratory or a working station with lab materials and equipment solely for pre-amplification such as pipettes, pipette tips, PCR tubes, 1.5 mL microcentrifuge tubes, tube racks, PPE, etc.
- The amplified DNA products should be stored separately from the pre-amplification reagents to avoid cross contamination. Other downstream analysis or treatment, such as DNA purification, preparation before sequencing, should be processed in another laboratory.

2. **Preparation of Controls**

- Positive Control: Dilute the concentration of the genomic DNA to 30 pg/µL with nuclease-free water. Add 1 µL of the 30 pg/µL genomic DNA solution to 4 µL of Cell Lysis Buffer in a PCR tube or well.
- Negative Control: Add 1 µL nuclease-free water to 4 µL of Cell Lysis Buffer in a PCR tube or well.

PROTOCOL

Note:

1. All reactions described in the procedures of MALBAC Single Cell WGA Kit take place in the same tube where the single cell has been isolated and lysed.
2. To avoid accidental removal of the cell (or parts of the cellular genome), carefully add an appropriate amount of reaction mix onto the inner wall of the tube, without disturbing the liquid.
3. Briefly centrifuge the mixture afterwards (2000rpm, 3 to 4 seconds). Transfer Cell Lysis Enzyme, Pre-Amp Enzyme and Amp Enzyme tubes to ice just before use, and other components can be thawed on ice before use.

Step 1: Cell Lysis

1. Prepare the Cell Lysis Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Cell Lysis Buffer	5 µL*（N+1）
Cell Lysis Enzyme	0.5 µL*（N+1）
Total Volume	5.5 µL*（N+1）

Note: N denotes the number of reaction.

2. Transfer samples into 5 µL of the prepared Cell Lysis Reaction Mix in a PCR tube or well (The volume of PBS solution carried over with the sample should not exceed 2 µL). Centrifuge briefly. Do not vortex.
3. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	50℃	50 min
	80℃	10 min
	4℃	Forever

Step 2: MALBAC Pre-Amplification

4. Prepare the MALBAC Pre-Amp Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Pre-Amp Buffer	30 µL*（N+1）
Pre-Amp Enzyme	1 µL*（N+1）

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Total Volume	31 μL*（N+1）

5. Add 30 μL of freshly-prepared MALBAC Pre-Amp Reaction Mix to each 5 μL of completed lysed samples and control groups(total volume should be 35 μL). Mix by vortexing and centrifuge briefly.
6. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	94℃	3 min
8	20℃	40 sec
	30℃	40 sec
	40℃	30 sec
	50℃	30 sec
	60℃	30 sec
	70℃	4 min
	95℃	20 sec
	58℃	10 sec
1	4℃	Forever

Step 3: Exponential Amplification

7. Prepare the Amp Reaction Mix according to the following table and thoroughly mix.

Component	Volume
Amplification Buffer	30 μL*（N+1）
Amp Enzyme	0.8 μL*（N+1）
Total Volume	30.8 μL*（N+1）

8. Add 30 μL of freshly-prepared Amp Reaction Mix to 35 μL of the Pre-Amp products(total volume should be 65 μL). Mix by vortexing and centrifuge briefly.
9. Incubate the sample(s) in a thermal cycler with heated lid using the following parameters tabulated below.

Cycle	Temperature	Time
1	94℃	30sec
17*	94℃	20sec
	58℃	30sec
	72℃	3 min
1	4℃	Forever

* Cycle number can be optimized according to sample types. 14 cycles is recommended for 100 pg of gDNA or equivalent amount . 17 cycles is recommended for a single cell sorted by flow cytometry. 19 to 21 cycles is recommended for single chromosome. For other types of samples, optimization of cycle number is suggested.

10. When the thermal cycling program is done, the amplified product can be stored at -20℃ for later use.

PRODUCT TECHNICAL SPECIFICATION

1. **Agarose Gel Electrophoresis :** Run 5 μL of the amplification product on a 1% agarose gel (110V, 25-35 min). A DNA smear ranging from 300 to 2000 bp will be observed.
2. **Quantification :** Purify the amplified DNA product and quantify it. The final yield weighs 2～5 μg.

LITERATURE REFERENCES

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Sequencing for 24-chromosome Aneuploidy Screening of Cleavage-stage Embryos. Fertil. Steril., 2014,Dec;102(6):1685-91.

TROUBLESHOOTING GUIDE

Issue	Potential Cause	Solution
No amplified product	Sample loss during embryo biopsy	Redo the embryo biopsy and make sure the biopsy samples are transferred completely to the cell lysis buffer. Avoid accidental removal of the genetic materials.
	Sample containing polymerase inhibitors	Polymerase inhibitors carried over with the starting materials can sometimes cause low amplification efficiency. Cell washing is strongly recommended to minimized no-cellular contamination. 1X PBS solution that contains no Ca^{2+}, Mg^{2+}, Mo^{2+} or heparin can be used for washing.
	Inactive enzymes	All components should be stored at -25～-15℃. All enzymes and buffers should be freshly prepared and thoroughly mixed before use. Avoid repeated freeze/thaw cycles for all enzymes and buffers.
Low amplified product	Sample containing polymerase inhibitors	Polymerase inhibitors carried over with the starting materials can sometimes cause low amplification efficiency. Cell washing is strongly recommended to minimized no-cellular contamination. 1X PBS solution that contains no Ca^{2+}, Mg^{2+}, Mo^{2+} or heparin can be used for washing. The volume of the washing buffer carried over to the cell lysis step should not exceed 1 μL in order to obtain optimal amplification efficiency.
	Degradation of genomic DNA	Avoid DNA degradation due to the improper cell storage or template preparation.
Amplified products in negative (no-template) control	Reagents contaminated by exogenous DNA	Keep kit reagents and the amplified DNA at designated storage space. Aliquot the reagents after the first use. Use sterile and nuclease-free tubes and filter tips to set up the reactions.
	Work area contaminated by external DNA	Clean the work space thoroughly by DNA and RNA decontamination reagents.
	Negative control groups contaminated by external DNA	Use new negative control groups.

NOTES

1. Check all the samples carefully. Exclude the contaminated sample(s).
2. Direct contact with skin or eyes may cause minor injury. Wear personal protection equipment including lab coat, disposable gloves and face mask when handling the sample(s) and the reagent(s). Properly dispose of all the goods which have contact with the sample after sterilization. Avoid the contamination of cells shed from the operator.
3. Buffer should be mixed thoroughly before use and repeated freeze/thaw should be avoided.
4. All the materials used must be dried and clean for contamination control. The consumables used during the experiment are for one-time use and cannot be reused.
5. The operator should be given professional training and perform the experiment according to the manual strictly.
6. The sample(s) should be treated as biohazard and be disposed of as a source of infection after experiment.
7. Minimize the influence of DNA contamination and enzyme inhibitors during sample preparation.
8. Avoid DNA degradation caused by improper storage or preparation.
9. Please store the components of the kit strictly as the instruction of the manual to avoid loss of effect or other adverse consequence caused by incorrect storage.
10. The kit is only for research use, not for diagnosis and other purposes.
11. The kit should be used strictly as the content of the manual. Yikon Genomics Co., Ltd. will take no

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responsibility caused by improper or incorrect uses unless otherwise specified by relative regulations and laws.

MANUFACTURE BASIC INFORMATION

Registration Unit/Manufacture: Yikon Genomics(Shanghai) Co., Ltd
Address:
Shanghai Office: Room 102, No.1, Lane 888, Tianlin Road, Hi-Tech Building, Shanghai Business Park 1, Caohejing Development Zone, Shanghai, 200233, China
Beijing Office: C bl℃k 3 layer 302, Yongfeng industrial base, Cheng Wing Road, No. 2 Building, No. 1 hospital green building, Haidian District, Beijing, 100094, China
Jiangsu Office: 5th Floor, Building TQB, No.1 China Medical City Avenue, Taizhou, Jiangsu, 225300, China
Suzhou Office: Room 201, No. B7, SIP BioBay, 218 Xinghu Road, Suzhou Industrial Park, Suzhou, Jiangsu, 215000, China
E-mail: technical@yikongenomics.com (technical support)
info@yikongenomics.com (consultation)
order@yikongenomics.com (order)
Tel: +86-10-5091-7399 (International, HongKong, Macau and Taiwan), 400-688-9230 (Mainland China)
Fax: +86-10-5091-7374
Web: www.yikongenomics.com

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