MALBAC® Platinum Single Cell RNA Amplification Kit Manual

PRODUCT NAME

MALBAC® Platinum Single Cell RNA Amplification Kit

SIZE / CAT. NO.

Size	Cat. No.
24 rxns	KT110700724
96 rxns	KT110700796

PRODUCT DESCRIPTION

The MALBAC® Platinum MicroRNA Amplification Kit can complete the reverse transcription of mRNA from 1 to 2000 cells or 10 pg to 20 ng of extracted eukaryotic total RNA within 5-6 hours. A linker sequence with a quantitative molecular tag was added to the 3' end of the cDNA using the MALBAC® Template-switching patented technique. Subsequent PCR amplification by this linker sequence yields a full-length cDNA amplification product, which effectively avoids 3' end bias and contamination of genomic DNA and rRNA during cDNA synthesis. The expression of quantitative molecular tags can be used for calculation of gene expression, retaining chain source information and fully amplifying mRNA sequence information. In general, 2-50 ng of high quality full-length double-stranded cDNA can be amplified in one reaction depending on the amount of input.

The reverse transcription and amplification success rate of this kit can reach to more than 95%. The full-length cDNA amplification products are seamlessly compatible with mainstream sequencing platforms, such as Illumina and Ion sequencers. The sequencing data(2.5M reads) can cover over 90% gene expressions and the consistency is greater than 90%, with no significant bias in amplification and less sample input required.

INTENDED USES

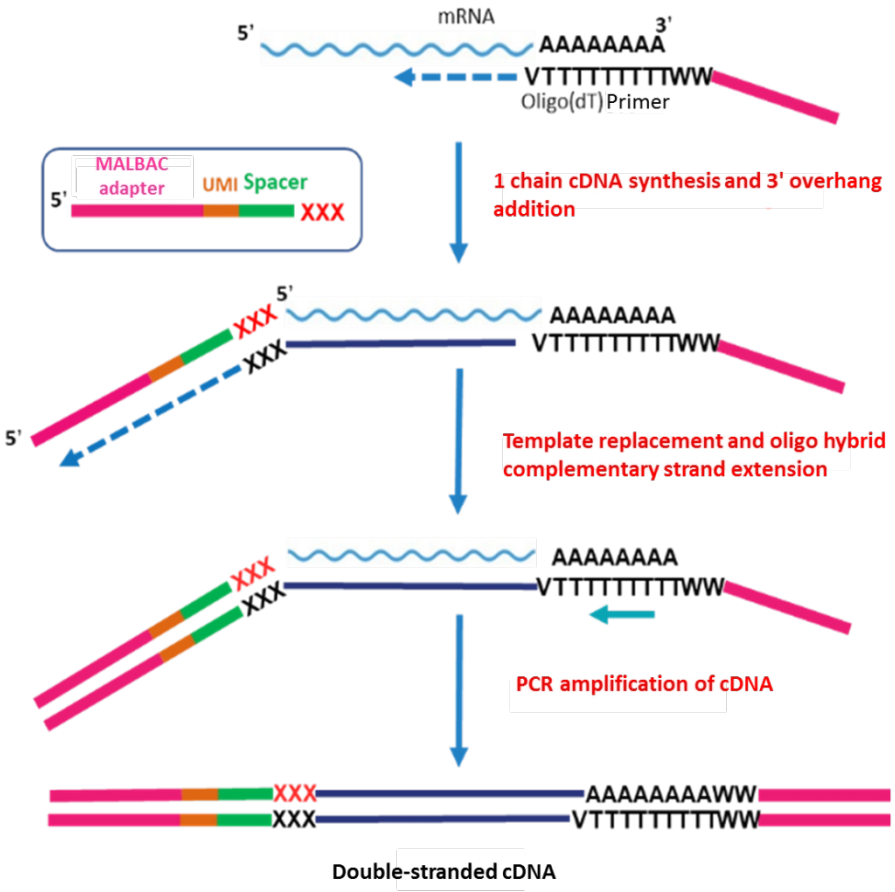
This product is mainly used for sample amplification of single-cell RNA-Seq. Single-cell RNA-Seq refers to the analysis of gene expression, functional enrichment and metabolic pathway in single-cell by reverse transcription and PCR amplification of single cell mRNA. This product can solve the problem of extremely low sample amount or heterogeneity which exists when using traditional RNA reverse transcription and amplification methods during the researches on early embryo development, stem cells, cancer, immunity, etc. . As a powerful tool in the studies of single-cell gene expressions, it has greatly expanded the range of applications for RNA-Seq, including:

1. Expression analysis of extremly low amount of samples (single-cell level).
2. Early embryo development.
3. Tumor cell heterogeneity .
4. Immune cell subpopulation.
5. Stem cell differentiation.

BASIC PRINCIPLE

This Product can achieve efficient and uniform amplification of full-length transcripts. Total RNA after heated lysis is released from single cell(or several single cells), of which the mRNA is reverse transcribed so cDNA is obtained. With MALBAC® Template-Switching patented technique, an adapter is added at the 3’ end of the cDNA and Template-switching is implemented. Then exponential amplification is performed using the adapter sequences as the anchors of the primers so that full-length cDNAs are produced which are used on downstream analysis or library preparation. The key patented technique of MALBAC® Template-switching guarantee the full amplification of mRNA sequences and meanwhile keep the source information of transcripts by allocating unique molecular identifiers(UMI) on each transcripts, achieving absolute quantification of transcripts on single-cell level and providing sufficient materials for downstream experiments or analysis of RNA-seq.

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



Schematic Diagram of Reverse Transcription and Amplification Process by MALBAC® Platinum Single Cell RNA Amplification Kit

COMPONENTS

Component	Cap Color	Voume * Quantity	
		24 rxns	96 rxns
Cell Lysis Buffer	White	36 μL * 1	144 μL * 1
RT Buffer	Red	319.2 μL * 1	1276.8 μL * 1
RT Enzyme Mix	Red	40.8 μL * 1	163.2 μL * 1
PCR Mix	Purple	720 μL * 1	1440 μL * 2
RNase Free H_2O	Yellow	1mL * 1	1mL *1

Note: Do not mix use reagents with different lot No.

EQUIPMENT & CONSUMABLES SUPPLIED BY USERS

1. Reagent: 1×PBS Buffer.
2. Equipment: Thermal cycler, Micro spectrophotometer, Vortexer, Microcentrifuge.

STORAGE & PERIOD OF VALIDITY

Store Condition: -25～-15℃; avoid repeated freeze-thaw cycles. Allocating Reagents is suggested if multi-time uses are needed.

Period of Validity: 24 months; MFD / EXP: On the package.

PRODUCT FEATURES

1. Low amount of starting material: Single cell (or 10pg total RNA).
2. High efficiency: high-quality and full-length cDNA obtained by mRNA reverse transcription and amplification.
3. Simple: One-tube and 3-steps reaction, the whole process takes 5 to 6 hours.

RESEARCH AREAS

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This kit can be applied to RNA reverse transcription and amplification during the researches of early embryo development, stem cell, cancer, immunology, etc. and solves the problems of low amount of strating material and heterogeneity.

SAMPLE SPECIFICATION

1. **Sample Amount:** This kit is designed for the amplification of transcripts from single cell or few cells, and the RNAs with polyA tails is amplified using Oligo dT primers. It can be used for multiple sample types:
   a. 1 to 2000 mammalian cells, or other eukaryotic cells without cell wall.
   b. 10pg to 20ng purified total RNA (if using trace amount of RNA then RNase Inhibitor is recommended to added to the RNase-Free H_2O of the kit as the dilution buffer for RNA, the ration between RNase inhibitor and RNase-Free H_2O is 1:49.
   c. This kit is not applicable to prokaryotic cells and the cells fixed by paraformaldehyde.
2. **Collection Method:** Cells collected through FACS, laser capture microdissection(LCM), biopsy by pipetting, microfluid technique are suitable for transcripts amplification by this kit.
3. **Pre-treatment of Samples:** Due to the instant changes of gene expressions in cells, the differences of cell types, cell viabilities and cell cycle stages could significantly affect the final production of cDNAs. Please make sure the influences of collection methods to cell viablity. We recommend identification of cell viability after each collection because in most cases the degradation of RNA in dead cells can lead to failure of the amplification. The experiment shall be implemented instantly after confirming the cell viability because improper storage could lead to RNA degradation. Cell wash or rinse using 1×PBS buffer (free of Mg^{2+} and Ca^{2+}) is recommended prior to experiment to avoid external RNA contamination.

PREPARATION BEFORE EXPERIMENT

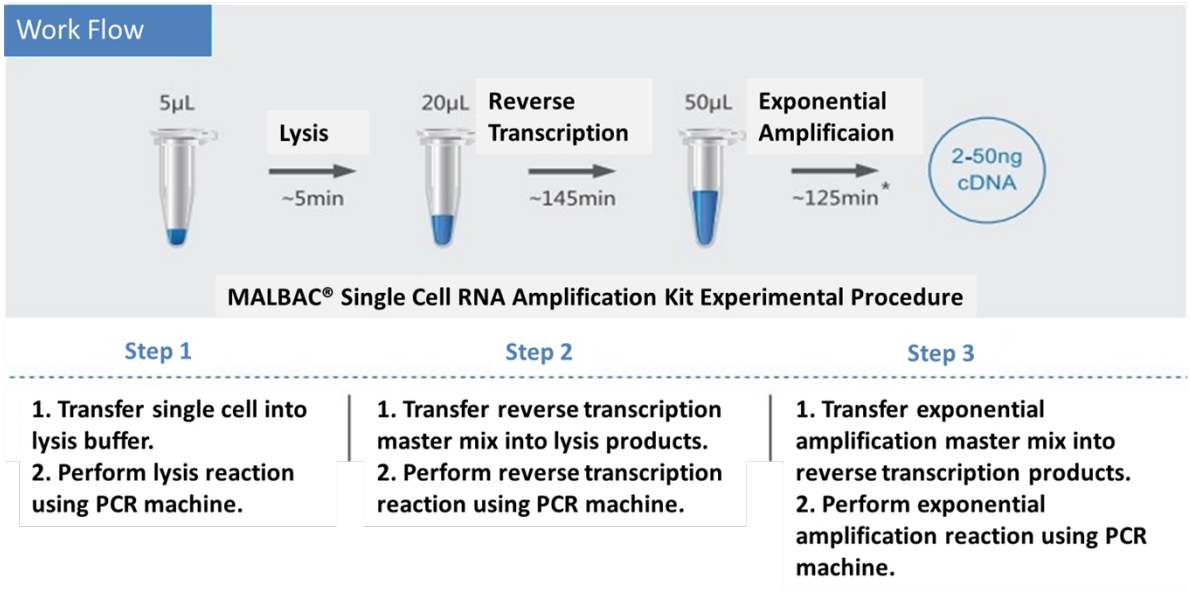
1. **Isolation of Pre-Amplification Sample Preparation Working Area**
   To aviod the external interference or RNase contamination, sample preparation shall be completed in an isolated room or area prior to the experiment. Dedicated equipment and experimental materials shall be prepared, including pipettors, pipette tips, PCR tubes, 1.5mL centrifuge tubes, tube racks, centrifuge machines, vortexers and lab coats.

   Please separate the storage compartments for amplification products and reagents. All the downstream processes shall be conducted in other lab(s).
2. **Preparation of Controls**
   Positive control: if using cells as starting material, 100 cells from the same batch(same treatment with the sample) can be used as positive control; if using nucleic acids as starting material, 1ng total RNA from the same batch(same treatment with the sample) can be used as positive control.

   Negative control: Adding 3.5μL RNase-Free H_2O to **1.5μL Cell Lysis Buffer** as negative control.

PROTOCOL

Summary of operation process



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Detailed operation process

Note:

1. Single cell reverse transcription and amplification are performed in the same PCR tube, and the volume of lysis reaction is small. Do not touch the liquid with pipette tip to avoid sample loss.
2. Carefully add reagent on the inner wall of the tube and no pipetting up/down is allowed to aviod generating bubbles.
3. Use pulse centrifugation (2000rpm for 3 to 4 seconds) to mix thoroughly.
4. Place the reagent containing enzyme in ice bath and thaw the reagent without enzyme on ice before use.

Step 1: Single cell (or few cells) collection and cell lysis

1. Dilution: dilute cells with 1x PBS (the following "PBS" is all refer to 1xPBS), centrifuge and resuspend to generate cell resuspension which containing around 20 cells/μL.
   a. Tissue treatment: homogenize tissue with liquid nitrogen, add 600 μL PBS to each 20 to 30 mg of tissue. Centrifuge using 12,000 rpm (~13,400×g) for 1 minute to collect cell precipitation. Remove the supernatant and resuspend the precipitation with PBS.
   b. Single layer cultured cells: detach the cells form the petri dish and collect cells in a centrifuge tube. Centrifuge using 12,000 rpm (~13,400×g) for 1 minute to collect cell precipitation. Remove the supernatant and resuspend the precipitation with PBS.
   c. Cell suspension: centrifuge using 12,000 rpm (~13,400×g) for 1 minute to collect cell precipitation. Remove the supernatant and resuspend the precipitation with PBS.

   Note:

   （1） Remove the culture medium as much as possible because it may inhibit the cell lysis process and affect RNA quality.

   （2） Resuspend cells as completely as possible and inaccuracy of cells counting could influence the single cell isolation.
2. Cell lysis: transfer single cell into **1.5 μL Cell Lysis Buffer** in a PCR tube.
   Note: the volume of PBS containing single cell has to be less than 3.5 μL. Rigorous volume control (total volume is 5.0μL, supplement with RNase-Free H2O if the total volume is less than 5.0μL) is helpful for efficient reverse transcription.
3. Transfer **13.3 μL RT Buffer** into a new PCR tube.
4. Put the samples (lysis mixture) and RT Buffer (do not add RT Enzyme Mix in RT Buffer) separately in a pre-heated PCR machine and run the following program.

Temperature	Time
72℃	3 min
0℃	2 min

Step 2: Reverse transcription to obtain full-length double-stranded cDNA

5. Add **1.7 μL RT Enzyme Mix** to the RT Buffer from the last step (total volume is 15μL). Label as "RT Mix" on the tube. Mix it by flip and pulse centrifuge.
6. In each cell lysis product, add 15μL RT Mix and instantly place the tube on ice. Then put the tube into the pre-heated PCR machine and run the following program.

Cycle	Temperature	Time
1	42℃	90 min
10	50℃	2 min
	42℃	2 min
1	70℃	15 min
	12℃	Forever

Step 3: Exponential Amplification

7. Add **30 μL PCR Mix** to the reverse transcription product from the last step (total volume is 50μL). Mix thoroughly and put the tube in the pre-heated PCR machine. Run the following program.

Cycle	Temperature	Time
1	98℃	3 min
7～18*	98℃	20 sec
	68℃	15 sec

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	72℃	6 min
1	72℃	5 min
	12℃	Forever

*Cycle number references：

Total RNA amount	Cell number	Cycle
10～20ng	1000～2000	7～8
1ng	100	11～12
100pg	10	14～15
10pg	1	17～18

Note: the above data are the references for GM12878 cell line. Due to the difference of the RNA amounts of different cell types, please adjust the PCR cycle of exponential amplification if using different cell type. We recommend confirming optimal cycle number by setting gradient cycle numbers at first try.

Step 4: Purification

8. This kit uses the protection system of reverse transcriptase which induces the clump of common magnetic beads. Purification effect would be influenced by direct use of magnetic beads. We recommend the following combined purification methods:

8.1 Firstly use column-based purification to remove the protection system of reverse transcriptase: DNA Clean & Concentrator™-5（Cat. No: D4013）is recommended and elute the products in 50μL Elution Buffer. Other column-based kits with the same effects can also be alternatives.

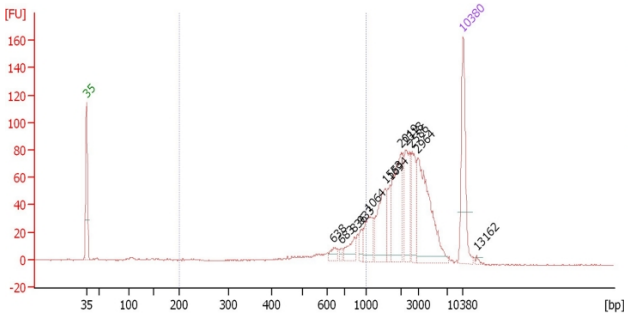
8.2 Then use magnetic beads to remove small-size fragments: subject the 50μL products from the last step to Agencourt AMPure XP beads（Cat. No: A63881）. Wash with ethnol as the manual and resolve the beads with 17.5μL Elution buffer. Revover 15 μL as the products and store properly. Other magnetic beads with the same effects can also be alternatives.

9. Store the purified product at -20℃ for later use.

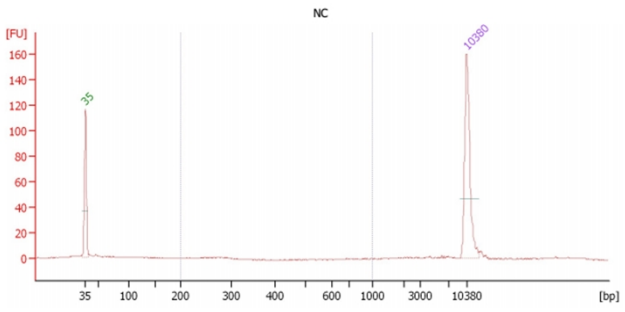
QUALITY ASSESSMENT

10. The yield and fragment distribution of the amplification products can be used as the indicators of quality assessment. It is recommended to use the Agilent 2100 Bioanalyzer for detection. 1 μL of the purified cDNA amplification product is appropriately diluted, uploaded on High Sensitivity DNA Chip and subjected to Agilent 2100 Bioanalyzer. For instructions, please refer to the manual of High Sensitivity DNA Chip.

Note: In general, depending on the amount of the starting templates, the reaction yields of the amplified product is betwwen 2ng and 50 ng. The size distribution of the purified cDNA amplification product is at 600-10000 bp, and the peak is located at 2500 bp. No distribution for negative control would appear. The following picture of the distribution is for your reference:



Size Distribution of Successfully Amplified Product



No Size Distribution of Negative Control

LIBRARY PREPARATION

The following two library preparation are recommended:

1. Transposase-based library preparation (read length and data volume are determined depends on practical requirements).
2. Fragmentation&adapter ligation-based library preparation (for absolute quantification of gene expressions, technical support of data analysis from Yikon is available).

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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 Reverse transcriptase inhibitor or RNase	It is recommended to clean the cells with a clean 1× PBS buffer before the experiment to ensure that the sample contains no inhibitors or RNase.
	Inactive enzymes	All components should be stored at -25～-15℃ except for the RNase water. The reagent reconstitution process needs to be carried out on ice, and it is divided before use to avoid repeated freezing and thawing.
Low amplified product	Sample containing Reverse transcriptase inhibitor or RNase	It is recommended to clean the cells with a clean 1× PBS buffer before the experiment to ensure that the sample contains no inhibitors or RNase.
	Degradation of RNA	Avoid cell or total RNA degradation due to the improper cell storage or template preparation, affecting amplification efficiency.
Amplified products in negative (no-template) control	Reagents contaminated by exogenous nucleic acids	Avoid kit components in contact with other amplification products or templates, and use reverse transcriptase and amplification with nuclease-free and nucleic acid-free laboratory consumables. It is recommended to clean the cells with a clean 1× PBS buffer before the experiment. And ensure that the experimental environment does not contain foreign RNA, DNA contamination.
	Work area contaminated by external nucleic acids	Clean the work space thoroughly by DNA and RNA decontamination reagents.
	Negative control groups contaminated by external nucleic acids	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 RNA contamination and polymerase inhibitors during sample preparation.
8. Avoid RNA 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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地址：上海市奉贤区望园路1698弄聚惠领域26号

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 block 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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