武汉昌美生物
Wuhan Charm Biotech

Charm-Pure™ Large-Scale Saliva Genomic DNA Purification

Store at room temperature, store Proteinase K and RNase A at 4 ºC

Product Contents and Storage

The components included with the Charm-Pure™ Large-Scale Saliva gDNA Purification are listed below. Upon receipt store all components at room temperature with the exception of Proteinase K and RNase A, which need to be stored at 4 ºC. Sufficient reagents are included to perform 25 or 125 genomic DNA extractions.

.Product Catalogue	JM--279-M	JM-279-L
Purification Scale	25	125
Binding Tube (BT4)	25 tubes	125 tubes
Lysis Buffer (PKLB)	11.0 ml	55.0 ml
Binding Buffer (KB6)	22.0 ml	110.0 ml
Spin-Column with Collection Tube (CC1)	50 sets	250 sets
Binding Buffer (GB5)	22.0 ml	110.0 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	11.0 ml	55.0 ml
Elution Buffer (EB2)	50.0 ml	250.0 ml
Proteinase K (PK2) (20 mg/ml)	2.2 ml	11.0 ml
RNase A (RA2) (20 mg/ml)	2.2 ml	11.0 ml

Product Description

The Charm-Pure™ Large-Scale Saliva Genomic DNA Purification kit is designed and optimized for easy and fast isolation of high-quality gDNA from 4 ml fresh saliva, frozen saliva or saliva sample stored in the Charm-Protect Saliva Collection and Storage Vial or preservation solutions from other companies. Based on Charm Biotech developed Solid Surface Reversible Binding (SSRB) technology and special silica membrane technology, 10 ML tubes coated with proprietary turbo-binders and spin-column with modified silica membranes are used to selectively capture and efficiently bind genomic DNA from saliva cell lysates. High-yield and high-quality DNA can be easily obtained from 4 ML saliva mixtures. The purified gDNA can be eluted in Elution Buffer or water. The purified ready-to-use gDNA is ideal for downstream application such as restriction digestion, primer extension, SNP analysis, PCR-free sequencing, whole genome amplification (WGA). DNA database/databank, and DNA methylation analysis.

Additional Materials Needed

- 100 % ethanol , Isopropanol
- 1.5 ml or 2.0 ml microtubes, 15 ml Falcon conic centrifuge tube
- Bench-top centrifuge capable of spinning 1.5 – 2.0 ml microtubes, Vortex or Shaker, Incubator (air or water) or hot blocker
- Swinging-bucket centrifuge capable of spinning 15 ml tube, such as Eppendorf Centrifuge 5804, Beckman GS-6, or Sovall 6600

General Precautions

- This kit is for research use only. All due care and attention should be exercised in the handling of the kits.
- Wear a laboratory coat, disposable gloves, and eye protection when handling reagents and tubes. Avoid ingestion and inhalation of reagents. Avoid skin contact with reagents in the kit. In case of contact, wash thoroughly with water. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. All MSDS information is available upon request.
- Always use proper aseptic techniques to avoid nuclease contamination when working with nucleic acids and use only sterile, new pipette tips to prevent cross contamination.

Preparation before Starting

- Prepare fresh working Binding Buffer prior to performing gDNA isolation from saliva lysates based on the number of samples processed. To make working Binding Buffer (KB6), mix 100 µl of Binding Buffer (KB6) with 400 µl 100 % Isopropanol and mix well. Prepare a master Binding Buffer solution based on (1) the number of samples processed and (2) any anticipated loss, generally 10 %, during dispensing. For working Binding Buffer (KB6), dispense 100 µl of Binding Buffer containing Isopropanol per 100 µl cell lysates (1:1 ratio). Discard the unused Binding Buffer at the end of the day.
- For JM-279-M kit, add 22 ml of 100 % ethanol to Washing Buffer I (WB1) and mix well. For JM-279-L kit, add 110 ml of 100 % ethanol to Washing Buffer I (WB1) and mix well. (Mark bottle that ethanol has been added!) – Store at room temperature and use WB1 containing ethanol within six (6) months.
- For JM-279-M kit, add 44 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For JM-279-Lkit, add 220 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. (Mark bottle that ethanol has been added!) – Store at room temperature and use WB2 containing ethanol within six (6) months.

Experimental Procedure

Preparing Lysates

The protocols listed below are for purification of gDNA from 4 ml of saliva preservation mixture. Adjust volume accordingly for actual volume of saliva mixture.

Note: If the saliva samples are stored in the Charm-Protect Saliva Preservation Solution, please follow the procedure below to prepare the saliva lysates. (If you need to isolate gDNA from saliva samples without using Charm-Protect Saliva Preservation Solution, please check "Experimental Procedure for Non-Charm Protected saliva samples").

1. Add 80 µl RNase A (RA2) into a 15 ml (Falcon) conic centrifuge tube.
2. Mix the saliva sample mixture stored in the Charm-Protect Saliva Preservation Solution in the Saliva Collection Vial by inverting and shaking 7-8 times. Let the vial or tube sit for 3-5 minutes.
3. Transfer 4 ml saliva preservation mixture from the Saliva Collection Vial into the 15 ml tube preloaded with RNase A (RA2) and mix well by inverting the tube 7 – 8 times after closing the cap. (Note: Don't collect the particulate settlement at the bottom of the collection vial when aspirate saliva mixture.)
4. Add 80 µl Proteinase K (PK2) into the tube and mix well by inverting the tube 15 – 18 times after closing the cap..
5. Incubate the conic tube at 62 ºC in an air (or water) incubator or a hot-block for 45 minutes with occasional mixing by tapping or inverting the tube. (Optional: Incubate the sample overnight at 52 ºC if prefer)
6. Centrifuge the conic tube at ⩾ 2,250 x g for 10 minutes to remove any particulate materials, such as food particles, undigested dead cells and fibers debris.

Experimental Procedure for Non-Charm Protected saliva samples

If your saliva samples are fresh saliva, frozen saliva or saliva mixed with saliva preservation solution from other companies, please prepare saliva lysates as follows:

1. Add 80 µl RNase A (RA2) into a 15 ml (Falcon) conic centrifuge tube.
2. Add 400 µl Lysis Buffer (PKLB) into the tube（the volume ratio of PKLB vs. saliva sample is 1 to 9）.
3. Mix the saliva sample by inverting the saliva container 7-8 times and then let the container sit for 3-5 minutes.
4. Transfer 3.6 ml saliva or saliva mixture into the conic tube preloaded with RNase A (RA2) and PKLB. Mix well by inverting the 15 ml tube 9 – 10 times after closing the cap. (Note: Don't collect the particulate settlement at the bottom of the container when aspirate saliva mixture.)
5. Add 80 µl Proteinase K (PK2) into the tube and mix well by inverting the tube 15 – 18 times after closing the cap.
6. Incubate the tube at 62 ºC in an air (water) incubator or a hot-blocker for 60 minutes with occasional mixing by tapping or inverting the tube. (Optional: Incubate the sample overnight at 52 ºC if prefer)
7. Centrifuge the 15ml tube at ⩾ 2,250 x g for 10 minutes to remove any particulate materials, such as food particles, undigested dead cells and fibers debris.

Binding Products

1. Transfer 3.9 ml clarified saliva lysate into a Binding Tube (BT4) without collecting the debris pellet at the bottom of the 15 ml tube (Note: To avoid the debris pellet, you may leave about 150-260 µl solution in the conic tube when doing aspiration).
2. Add 3.9 ml working Binding Buffer (KB6) containing Isopropanol to the Binding Tube (BT4). Mix well by inverting the Binding Tube 15 – 18 times after closing the cap to obtain a homogenous solution. (Note: It is crucial to mix working Binding Buffer with saliva lysate thoroughly.)
3. Centrifuge the Binding Tube (BT4) at ⩾ 2,250 x g at room temperature for 15 minutes to bind the DNA.
4. Open the cap,remove the solution from the Binding Tube by flipping the Binding Tube over a waste container and shaking briskly. Let the tube stand upside down on a piece of clean absorbent paper for 3 – 8 minutes to drain as much liquid as possible.

5. Add 1.2 ml Elution Buffer (EB2) to the Binding Tube. Close the cap and vortex the tube for 1 minute and incubate the tube at 62 °C for 15 - 20 minute to dissolve the DNA pellet thoroughly. (You may vortex again after 62 °C incubation to help dissolve the pellet)
6. Add 720 µl Binding Buffer (GB5) to the Binding Tube and mix well by inverting the tube 8-9 times after closing the cap.
7. Add 720 µl ethanol to the mixture above and mix well by inverting the tube 12- 15 times (The total volume is about 2.64 ml)
8. Transfer two 660 µl solution mixtures from the step above to two Spin-Columns with Collection Tubes (CC1) separately. Centrifuge the Spin-Columns at 8000 rpm (RCF = 5800 g) for one minute to allow all liquid pass through the membrane at room temperature.
9. Discard the filtrate in the Collection Tube and reinsert the Spin-Column back into the Collection Tube.
10. Repeat step 8 and 9 above once to allow all solution mixture pass through the membrane.

Washing Products

1. Add 500 µl of Wash Buffer I (WB1) with ethanol to each Column.
2. Centrifuge the Spin-Column at maximum speed (≥ 13000 rpm or 16000 X g) for 1 minute at room temperature. Discard the filtrate.
3. Add 500 µl of Wash Buffer II (WB2) with ethanol to each Column.
4. Centrifuge the Spin-Column at maximum speed (≥ 13000 rpm or 16000 X g) for 1 minute at room temperature. Discard the filtrate.
5. Repeat step 3 and 4 above once for a total of two washes with Wash Buffer II (WB2).
6. Centrifuge the spin-column at maximum speed for one minute to remove any residual wash buffer with ethanol. Discard collection tube.
7. Insert the Spin-Column into a new clean micro-tube (self-provide). (Note: When transferring the spin-column, be careful of preventing the residual wash buffer contacting the tip of the column)

Eluting Genomic DNA

1. Warm up the Elution Buffer (EB2) in a 1.5 ml micro-tube with a 62 °C hot block or water-bath.
2. Add 150 µl Elution Buffer (EB2) onto the center of the Spin-Column, incubate the Spin-Column with collection micro-tube at 62 ºC in an air incubator or a hot-blocker for at least 5 minutes.
3. Centrifuge the column at maximum speed for 1 minute at room temperature. The micro-tube contains the filtrate which contains purified gDNA. (Note: You may perform a second elution with another 100 µl of Elution Buffer (EB2) if desired. This can increase the total gDNA yield by 15-30 %, but the final concentration of isolated DNA in the eluted sample is reduced because of the increased volume).
4. Combine the two eluted gDNAs from the same saliva sample. The eluted gDNA may be used immediately in downstream applications. Alternatively, the eluted gDNA may be stored in the micro-tube at 4 ºC for short-term storage or –20 ºC for long-term storage.

Electrophoresis and Downstream Application

Purified gDNA can be examined by agarose gel electrophoresis. Yield can be measured with a spectrophotometer or fluorescent DNA assays. The yield of purified gDNA varied in the range of 20 – 120 µg from different saliva samples， $A_{260/280}$ ⩾1.7 (the majority is between 1.8 – 2.0). Purified gDNA can be used immediately for downstream application, such as restriction enzyme digestion, primer extension, SNP analysis, PCR, STR analysis, DNA sequencing, whole genome amplification (WGA) and other molecular manipulations.

Troubleshooting

Problem	Cause	Solution
Low DNA yield	Poor quality of starting material	Be sure the protocol for sample collection and preservation recommended by manuals is followed correctly.
	Incomplete lysis	If incomplete lysis is observed, extend the incubation time at 62 ºC for an addition of 15 – 30 minutes.
	Variable DNA amount from different individual saliva samples	Increase the starting saliva sample volume for DNA isolation. Collect oral swap samples or more saliva samples
No PCR product	Missing Component in the PCR mixture	Be sure to add all components. Check positive control and negative control for PCR reaction.

Charm-Pure™精纯大量唾液 DNA提纯试剂盒

室温运输和保存，Proteinase K 和 RNase A 保存在 4 ºC。

试剂盒组成及贮存

Charm-Pure™ 精纯大量唾液DNA提纯试剂盒可从 4 ML 唾液中提纯高质量的gDNA，其组成如下表，除了 Proteinase K 和 RNase A 需要保存在 4 ºC 之外，其余组分室温保存即可。可进行 50或者250 次唾液gDNA 的提纯。

Product Catalogue	JM-279-M	JM-279-L
Purification Scale	25 次	125 次
Binding Tube (BT4)	25 个	125 个
Lysis Buffer (PKLB)	11.0 ml	55.0 ml
Binding Buffer (KB6)	22.0 ml	110.0 ml
Spin-Column with Collection Tube (CC1)	50 套	250 套
Binding Buffer (GB5)	22.0 ml	110.0 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	11.0 ml	55.0 ml
Elution Buffer (EB2)	50.0 ml	250.0 ml
Proteinase K (PK2) (20 mg/ml)	2.2 ml	11.0 ml
RNase A (RA2) (20 mg/ml)	2.2 ml	11.0 ml

产品介绍

Charm-Pure ™ 精纯大量唾液 DNA 提纯试剂盒专为从 4 ML 新鲜唾液、冻存唾液、Charm-Protect™ 或其他公司唾液保存液中分离提纯高质量的唾液 gDNA 而设计，操作步骤简单迅速。昌美生物将固体表面可逆结合技术（SSRB）和传统的核酸硅膜技术相结合，采用特别的结合管和改进的硅膜来选择性地吸附核酸，能有效处理 4 ml 的唾液混合液，高效率地提纯回收高纯度的唾液 gDNA。产量高，质量好！提纯的 gDNA 可直接用于下游各种实验，如限制性酶切反应、SNP、序列分析、DNA 建库，NGS、全基因组扩增（WGA）和 DNA 甲基化分析等。

亮点

操作简单：可处理 4 ml 唾液样品，避免多次处理小量样品，操作步骤简单，不需要特别的仪器。

质量可靠，回收率高：采用独特的固体表面可逆结合技术和改进的硅膜核酸结合技术，能最大程度的回收唾液样品的 gDNA 。质量高，重复性好。

所需的额外材料

- 无水乙醇、异丙醇
- 1.5 ml 或 2.0 ml 离心管，15 ml 的的可离心管。涡旋振荡器、温箱，水浴锅或恒温金属浴。
- 可离 1.5 ml 或 2.0 ml 离心管的离心机和可离心 15 ml 管的摇摆式离心机.

常规防范

- 该试剂盒仅用于实验。
- 实验操作时需穿实验服，戴一次性手套。避免摄取和吸入试剂，避免与试剂盒中试剂进行直接接触，一旦接触，用水彻底冲洗。
- 提纯核酸时，使用无菌新吸头避免污染。

实验前准备

- 在进行从唾液提纯 gDNA 之前，需要根据提纯的样品数目制备新鲜的 Binding Buffer（KB6）工作液： 将 Binding Buffer（KB6）与异丙醇以 1：4 体积比混匀，如配制 500 µl Binding Buffer（KB6）工作液需 将 100 µl Binding Buffer（KB6）与 400 µl 异丙醇混合均匀得到。需要制备 Binding Buffer（KB6）工作 液的体积根据：（1）待提纯的样品数目；（2）预期的损失，在悬浮过程中，一般会有 10%的损失。每 100 µl 细胞裂解液需加 100 µl 含异丙醇的 Binding Buffer（KB6）工作液（1：1 体积比）。剩余的 Binding Buffer（KB6）工作液当天予以丢弃。
- 制备 Washing Buffer I（WB1）工作液：如果是 JM-279-M 试剂盒，加 22 ml 无水乙醇到 Washing Buffer I（WB1）中，充分混匀。如果是 JM-279-L 试剂盒，加 110 ml 无水乙醇到 Washing Buffer I（WB1）中，充分混匀。在瓶上做好标记表示已加乙醇！室温保存，加乙醇的 WB1 在六个月之内使用。
- 制备 Washing Buffer II（WB2）工作液：如果是 JM-279-M 试剂盒，加 44 ml 无水乙醇到 Washing Buffer II（WB2）中，充分混匀。如果是 JM-279-L 试剂盒，加 220 ml 无水乙醇到 Washing Buffer II（WB2）中，充分混匀。在瓶上做好标记表示已加乙醇！室温保存，加乙醇的 WB2 在六个月之内使用。
- 该试剂盒该可从 4 ml 唾液保存液中提纯 gDNA（2 ml 唾液 + 2 ml 唾液保存液）或冻存的唾液。在收集唾液时候，为了避免 gDNA 降解，请遵守标准的 Charm-Protect™ 唾液 DNA 保存液收集和保存程序收集唾液。

操作指南

制备唾液细胞裂解液

注意：如果唾液样本是昌美生物 Charm-Protect™ 唾液保存液保存的唾液，请使用以下步骤制备唾液细胞裂解液（如果您需要提纯其它唾液样本， 请见“非昌美生物 Charm-Protect™ 唾液保存液保存的唾液操作步骤”。）

1. 取 80 µl RNase A（RA2）到一个 15 ml 离心小管中。
2. 将储存在 Charm-Protect 唾液收集瓶中的唾液样品与唾液保存液颠倒 7-8 次后静置 3-5 分钟，让颗粒杂物沉淀到瓶底。
3. 从唾液收集瓶中吸取 4 ml 唾液与保存液的混合物到预先加了 RNase A（RA2）的 15 ml 离心管中，将离心管盖子盖上后颠倒 7-8 次，混合均匀。当吸取唾液混合物时，注意不要吸到收集瓶底颗粒状沉淀杂物。
4. 向管中加 80 µl Proteinase K（PK2），将离心管盖紧后颠倒离心管 15-18 次，充分和唾液混合均匀。
5. 将离心管置于 62 ºC ，温箱或者水浴锅或金属浴中温育 45 分钟，进行裂解，期间颠倒混合离心管，如有需要，也可将样品在 52 ºC 温育过夜。（注意不是 62 ºC 过夜）。

6. 将离心管在转速 ≥ 2,250 x g 的摇摆式离心机里室温离心 10 分钟来去除颗粒物质，如食物颗粒、未经消化的死细胞和纤维碎片。

非昌美生物 Charm-Protect™ 唾液保存液保存的唾液操作步骤

如果您的样本是新鲜唾液、冰冻的唾液，或者是使用其他公司的唾液保存液保存的唾液，请使用以下步骤制备唾液细胞裂解液：

1. 取 80 µl RNase A（RA2）到一个 15 ml 离心小管中。
2. 向管中加入 400 µl Lysis Buffer （PKLB）（PKLB 和唾液样品的体积比例是 1: 9）。
3. 将唾液样品颠倒 7-8 次后静置 3-5 分钟，让颗粒杂物沉淀。
4. 从样本中吸取 3600 µl 唾液或者唾液混合物，加到预先加了 RNase A（RA2）和 PKLB 的 15 ml 离心管中, 将离心管盖子盖上后颠倒 9-10 次，混合均匀。（当吸取唾液时，注意不要吸到底部颗粒状沉淀杂物。）
5. 向管中加 80 µl Proteinase K（PK2），将离心管盖紧后颠倒离心管 15-18 次，充分和唾液混合均匀。
6. 将离心管置于 62 °C 温箱，水浴锅或金属浴中温育 60 分钟，进行裂解，期间颠倒混合离心管，如有需要，也可将样品放在 52°C 温育过夜 （注意不是 62 °C 过夜）。
7. 将离心管在转速 ≥ 2,250 x g 的摇摆式离心机里室温离心 10 分钟来去除颗粒物质，如食物颗粒、未经消化的死细胞和纤维碎片。

结合 DNA 产物

1. 转移 3900 µl 澄清的唾液裂解液到一个 10 ml 结合管（BT4）中，注意不要吸到离心管底的碎片沉淀。
2. 加 3900 µl 含有异丙醇的 Binding Buffer（KB6）工作液到结合管（BT4）中，将结合管（BT4）盖紧后颠倒结合管 15-18 次，充分混合均匀（注意：溶液充分混合非常重要）。
3. 将结合管（BT4）在转速 ≥ 2,250 x g 的摇摆式离心机室里温离心 10 分钟来结合 DNA 到管壁。
4. 直接将结合管中的液体倾倒入废液收集杯中，然后将结合管倒放在干净的吸水纸上面 3 至 8 分钟，使管内液体尽量去掉。
5. 加 1200 µl Elution Buffer（EB2）溶液到离心小管，涡旋 1 分钟，62 °C 温育 15 -20 分钟，再涡旋 1 分钟，使沉淀完全溶解。
6. 加 720 µl Binding Buffer（GB5）到结合管内，将结合管（BT4）盖紧后颠倒结合管 7 - 8 次，混合均匀。
7. 向上述混合物中加 720 µl 乙醇，将管盖紧后颠倒结合管 15-18 次，充分混合均匀。（总体积是 2640 µl）
8. 将 660 µl 上述制备的混合物转移到配有收集管（Collection Tube）的离心柱（Spin-Column）中，共转移 2 次，分别到 2 套离心柱。室温下 8000 rpm（RCF = 5800 *g*）离心 1 分钟，让所有液体通过离心柱。
9. 取出 Spin-Column，将收集管中的滤液丢弃，将 Spin-Column 重新插回到收集管中。
10. 重复上述 8 和 9 步骤， 将全部溶液都通过 2 个离心柱。

清洗 DNA 产物

1. 向离心柱（Spin-Column）中加 500 µl 含有乙醇的 Wash Buffer I（WB1）。
2. 将带有收集管的离心柱（Spin-Column）以最大转速（≥13000 rpm 或 16000 *g*）室温离心 1 分钟，倒掉收集管中滤液。
3. 向 Spin-Column 中加 500 µl 含有乙醇的 Wash Buffer II（WB2）。

4. 将 Spin-Column 以最大转速（≥13000 rpm 或 16000 *g*）室温离心 1 分钟，倒掉收集管中滤液。
5. 重复步骤 3 和 4，即用 Wash Buffer II（WB2）洗两次。
6. 以最大转速将带有收集管的 Spin-Column 离心 1 分钟来彻底去除含有乙醇的 WB2。丢掉收集管。将 Spin-Column 放入一个干净的 1.5 ml 离心管中。（在转移 Spin-Column 时，注意避免残留的 Wash buffer 接触到 Spin-Column 的尖端。）

gDNA 的洗脱

1. 根据需要洗脱的样品数量，取一定体积的 Elution Buffer（EB2）到 一个 1.5 ml 离心管中，置于 62 °C 金属浴 或水浴中加热。
2. 向 Spin-Column 的膜中央，加 150 µl 预热的 Elution Buffer（EB2），将带有离心小管的小柱置于 62 °C 金属浴温育至少 5 分钟。
3. 室温下最大速度离心 1 分钟，小柱下 1.5 ml 离心管中的滤液即为纯化的 gDNA。注意：如果有需要，可另加 100 µl Elution Buffer（EB2）进行再次洗脱。这一步可增加产量 15 - 30 %，但提纯的 gDNA 因为体积增大，终 浓度会降低。
4. 将来源于同一唾液样本洗脱的 DNA 样本合并。洗脱到离心管中的 gDNA 可直接用于各种下游反应，或者置于 4 °C 短期保存或 - 20 °C 长期保存。

电泳及下游的应用

提纯的 gDNA 可用琼脂糖凝胶电泳进行质检，也可用分光光度计或荧光标记法测定产量。根据不同的唾液来源，gDNA 产量在 20 µg - 120 µg 之间，$A_{260/280}$ 比值≥1.7，大部分值在 1.8 - 2.0 之间。纯化的 gDNA 可直接用于下游反应，如限制性酶切反应、SNP、STR，DNA 序列分析、DNA 建库，全基因组扩增（WGA）及其它分子实验操作。

常见问题及解决方案

问题	原因	解决方案
DNA 产量低	起始材料质量差。	确保正确遵守手册推荐的样品收集和保存程序。
	裂解不彻底	将 Proteinase K 消化的时间延长 15-30 分钟
	样品本身 DNA 量不同（唾液 DNA 含量，因不同个体体质而异，如小孩唾液 DNA 量比老年人少）	增加要提纯唾液样品起始量，采集口腔拭子或者更多的唾液
PCR 没有产物	PCR 反应混合物中漏加组分	确保加了所有的组分。核对 PCR 反应的阳性对照和阴性对照。