武汉昌美生物
Wuhan Charm Biotech

Charm-Pure™ Genomic DNA Purification from Saliva (Spin- Column)

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

Product Contents and Storage

The components included with the Charm Pure™ gDNA Purification from saliva 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 50 or 250 sample gDNA extractions.

Product Catalogue	CT-236-M	CT-236-L
Purification Scale	50	250
Spin-Column with Collection Tube (CC1)	50	250
Lysis Buffer (PKLB)	1.65 ml	8.25 ml
Binding Buffer (GB3)	8.5 ml	42.5 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	11.0 ml	55.0 ml
Elution Buffer (EB2)	5.5 ml	27.5 ml
Proteinase K(PK2) (20 mg/ml)	0.55 ml	2.75 ml
RNase A (RA2) (20 mg/ml)	0.55 ml	2.75 ml

Product Description

The Charm-Pure™ g DNA Purification from Saliva Kit is designed and optimized for easy and fast isolation of high-quality gDNA from fresh saliva, frozen saliva or or saliva sample stored in the Charm-Protect Saliva Collection and Storage Vial or preservation solutions from other companies. Charm-Pure™ system employs modified silica membranes as selective nucleic acid captures in a Spin-Column format to efficiently extract cell gDNA from saliva with higher yield and purity than conventional silica membrane. DNA molecules can specifically interact with the modified silica membrane in the extraction buffer, while proteins and other contaminants are not bound and remain in the solution and passed through. After washing the column with washing buffer to remove unbound material, the purified DNA can be easily 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, sequencing, whole genome amplification (WGA) and DNA methylation analysis.

Additional Materials Needed

- 100 % ethanol
- Isopropanol
- 1.5 ml or 2.0 ml microtubes
- Pipettors and pipet tips
- Bench-top centrifuge capable of spinning 1.5-2.0 ml microtubes
- Vortex or Shaker
- Incubator (air or water) or hot block

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

- For CT-236-M kit, add 22 ml of 100 % ethanol to Washing Buffer I (WB1) and mix well. For CT-236-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 CT-236-M kit, add 44 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For CT-236-L kit, 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.
- Please following the standard Charm-Protect Saliva DNA Preservation Solution collection and preservation procedure to minimize sample degradation before proceeding gDNA purification procedure in the current protocol.

Experimental Procedure

Preparing Lysates

The protocols listed below are for purification of gDNA from 300 µl of saliva preservation mixture.For purification of 600 µl of saliva preservation mixture, please call us for details.
Note: If the samples are Charm-Protect saliva, prepare lysates as follows (if you need to isolate other saliva samples, please see "Procedure for Non-Charm-Protected Saliva".

Procedure for saliva with Charm-Protect saliva preservation solution:

1. Add 10 µl RNase A (RA2) into a 1.5 ml or 2.0 ml microtube.
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 sit for 3-5 minutes.
3. Transfer 300 µl saliva preservation mixtures from the Saliva Collection Vial into the microtube preloaded with RNase A (RA2) and mix well by pipetting the solution 8-9 times or by inverting the microtube 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 10 µl Proteinase K (PK2) into the saliva mixture and mix well by inverting the microtube 15 -18 times after closing the cap.
5. Incubate the microtube at 62 ºC in an air incubator or a hot-blocker for 45 minutes with occasional mixing by tapping or inverting the tube. (Optional: Incubate the sample overnight at 52 ºC if prefer)

Procedure for Non-Charm-Protected saliva:

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

1. Add 10 µl RNase A (RA2) into a 1.5 ml or 2.0 ml microtube.
2. Add 30 µl Lysis Buffer (PKLB) (the volume ratio of PKLB and saliva sample is 1to 9).
3. Transfer 270 µl saliva or saliva preservation mixture into the microtube preloaded with RNase A (RA2) and PKLB above and mix well by pipetting the solution 8-9 times or by inverting the microtube 7-8 times after closing the cap.
4. Add 10 µl Proteinase K (PK2) into the saliva mixture and mix well by inverting the microtube 15 -16 times after closing the cap.
5. Incubate the microtube at 62 ºC in an air incubator or a hot-blocker for 45 minutes with occasional mixing by tapping or inverting the tube. (Optional: Incubate the sample overnight at 52 ºC if prefer.)

Binding DNA

1. Add 150 µl Binding Buffer (GB3) into the saliva mixture, and add 200 µl isopropanol into the saliva mixture and mix well by pipetting the solution 8-9 times
2. Transfer all saliva solution mixture (about 670 µl) from step above to a Spin-Column with a Collection Tube (CC1).
3. Centrifuge the Spin-Column at 8000 rpm (RCF = 5800 g) for 1 minute at room temperature. Discard the filtrate in the Collection Tube and reinsert the Spin-Column back into the Collection Tube.

Washing DNA

1. Add 500 µl of Wash Buffer I (WB1) with ethanol to the Column.
2. Centrifuge the Spin-Column at maximum speed (≥ 13000 rpm or 16000 g) for 1 minute at room temperature. Discard the filtrate.

3. Add 500 µl of Wash Buffer II (WB2) with ethanol to the Column.
4. Centrifuge the Spin-Column at maximum speed (≥ 13000 rpm or 16000 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. And reinsert the Spin-Column into a new clean microcentrifuge 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 the hot 50 µl Elution Buffer (EB2) into the center of the Spin-Column and then incubated them at 62 ºC for 3-5 minutes.
3. Centrifuge the column at maximum speed for 1 minute at room temperature. The microtube contains the filtrate which contains purified gDNA. (Note: You may perform a second elution with another 50 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. The eluted gDNA may be used immediately in downstream applications. Alternatively, the eluted gDNA may be stored in the microtube 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, fluorescent DNA assays or other quantification methods. For electrophoresis, loading 5-8 µl purified DNA is recommended. The purified DNA is suitable for use in restriction 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.
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™唾液 gDNA 提纯试剂盒（离心柱法）

室温保存，Proteinase K 和 RNase A 保存在 4 ºC。

试剂盒组成及贮存

Charm-Pure™ 唾液 DNA 提纯试剂盒可从唾液中提纯 gDNA，其组成如下表所示。所有的组分除了 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 gDNA 的提纯。

Product Catalogue	**CT-236-M**	**CT-236-L**
Purification Scale	50 次	250 次
Spin-Column with Collection Tube (CC1)	50 套	250 套
Lysis Buffer (PKLB)	1.65 ml	8.25 ml
Binding Buffer (GB3)	8.5 ml	42.5 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	11.0 ml	55.0 ml
Elution Buffer (EB2)	5.5 ml	27.5 ml
Proteinase K(PK2) (20 mg/ml)	0.55 ml	2.75 ml
RNase A (RA2) (20 mg/ml)	0.55 ml	2.75 ml

产品介绍

Charm-Pure™ 唾液 gDNA 提纯试剂盒专为从新鲜唾液、冰冻唾液或者 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。Charm-Pure™ 提纯系统离心柱（Spin-Column）采用改进的硅膜来选择性地吸附核酸，较常规的硅膜更加高效地提纯高纯度的唾液 gDNA。抽提缓冲液中的 DNA 分子可特异性地与改进后的硅膜相结合，而蛋白和其它污染物不被结合而保留在溶液中透过硅膜。用 Washing buffer 来清洗离心柱（Spin-Column），去除未结合的物质，提纯的 gDNA 可用 Elution Buffer（EB2）或水来洗脱。提纯的 gDNA 直接可用于下游反应如限制性酶切反应、SNP 分析、PCR、序列分析、全基因组扩增（WGA）和 DNA 甲基化分析等。

所需的额外材料

- 无水乙醇
- 异丙醇
- 1.5 ml 或 2.0 ml 离心管
- 移液器和吸头
- 可离 1.5 ml 或 2 ml 离心管的离心机
- 涡旋振荡器
- 水浴锅或恒温金属浴

常规防范

1. 该试剂盒仅用于实验。
2. 实验操作时穿实验服，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皮肤接触，一旦接触，用水彻底冲洗。
3. 提纯核酸 DNA 时，使用无菌新吸头避免污染。

实验之前准备

1. CT-236-M 试剂盒，加 22 ml 无水乙醇到 Washing Buffer I（WB1）中，充分混匀。CT-236-L 试剂盒，加 110 ml 无水乙醇到 Washing Buffer I，充分混匀。注意在瓶上做好标记表示已加乙醇！– 室温保存，加了乙醇的 WB1 在六个月之内使用。
2. CT-236-M 试剂盒，加 44 ml 无水乙醇到 Washing Buffer II（WB2）中，充分混匀。CT-236-L 试剂盒，加 220 ml 无水乙醇到 Washing Buffer II，充分混匀。注意在瓶上做好标记表示已加乙醇！– 室温保存，加了乙醇的 WB2 在六个月之内使用。
3. gDNA 提纯实验之前，为避免 gDNA 降解，请遵守标准的 Charm-Protect 唾液 DNA 保存液收集和保存程序。

操作指南

步骤一：制备裂解液

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

昌美生物 Charm-Protect ™ 保存液保存的唾液操作步骤：

1. 加 10 µl RNase A（RA2）到一个 1.5 ml 或 2.0 ml 的离心管中。
2. 将储存在 Charm-Protect 唾液收集瓶中的唾液样品与唾液保存液来回颠倒和震荡 7 - 8 次后静置 3 - 5 分钟。
3. 从唾液收集瓶中吸取 300 µl 唾液保存混合液到加了 RNase A（RA2）的离心管中，吹打溶液 8 - 9 次或盖上盖子后颠倒离心管 7 - 8 次，混合均匀。
4. 加 10 µl Proteinase K（PK2）到唾液混合液中，盖上离心管盖子，颠倒离心管 15 - 18 次，充分混匀。
5. 将离心管置于 62 ºC 水浴锅或金属浴中温育 45 分钟，期间颠倒混合离心管。若有需要，也可将样品 52 ºC 温育过夜。

非昌美生物 Charm-Protect ™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 10 µl RNase A（RA2）到一个 1.5 ml 或 2.0 ml 离心管中。
2. 向管中加入 30 µl Lysis Buffer（PKLB）（PKLB 和唾液样品的体积比例是 1: 9）。
3. 吸取 270 µl 唾液或者唾液混合液到加了 RNase A 和 PKLB 的离心管中，吹打溶液 8 - 9 次或盖上盖子后颠倒离心管 7 - 8 次，混合均匀。
4. 加 10 µl Proteinase K（PK2）到唾液混合液中，盖上离心管盖子，颠倒离心管 15 - 18 次，充分混匀。
5. 将离心管置于 62 º C 水浴锅或金属浴中温育 45 分钟，期间颠倒混合离心管。若有需要，也可将样品 52 ºC 温育过夜。

步骤二：结合 DNA

1. 向上述唾液混合液中加 150 µl Binding Buffer（GB3）和 200 µl 异丙醇，来回吹打溶液 8 - 9 次，混合均匀。
2. 将所得的唾液混合液（约 670 µl）转移到含有收集管（Collection Tube）的离心柱（Spin-Column）中，室温下 8000 rpm（RCF = 5800 *g*）离心 1 分钟。
3. 取出 Spin-Column，将收集管中的滤液予以丢弃，将 Spin-Column 重新插入到收集管中。

步骤三：清洗 DNA

1. 向 Spin-Column 中加 500 µl Wash Buffer I（WB1）工作液（含有乙醇）。
2. 将带有收集管的 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。将 Spin-Column 重新插入到收集管中。
3. 向 Spin-Column 中加 500 µl Wash Buffer II（WB2）工作液（含有乙醇）。
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。将 Spin-Column 重新插入到收集管中。
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 Wash Buffer，丢掉收集管。将 Spin-Column 放入干净的 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。）

步骤四：洗脱 DNA

1. 取一定体积的 Elution Buffer（EB2）到 1.5 ml 离心管中，置于 62 °C 金属浴或水浴中加热。
2. 向 Spin-Column 膜中央加 50 µl 预热的 Elution Buffer（EB2），62 ºC 温育 3-5 分钟。
3. 室温下最大速度离心 1 分钟，1.5 ml 离心管中的滤液即为纯化的 gDNA。注意：如果有需要，可用 50 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. 洗脱到离心管中的 gDNA 可直接用于下游反应，或者置于 4 ºC 短期保存或 - 20 ºC 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。可用分光光度计或荧光标记法或其它定量方法来测量产量。建议取 5-8 µl 提纯的 DNA 进行电泳检测。纯化的 gDNA 可直接用于下游反应，如限制性酶切反应、引物延伸、SNP 分析、PCR、STR 分析、DNA 序列分析、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。 裂解不彻底。 样品本身量不同（统计发现，每个人的唾液 DNA 含量是不同的，小孩 DNA 量少，老年人 DNA 量多）。	确保严格遵守手册推荐的样品收集和保存程序。 62 ºC 温育时间延长 15-30 分钟。 增加提纯时唾液使用量，用 600 µl - 1200 µl 唾液保存液来提纯 gDNA，请来电咨询详细步骤。
PCR 没有产物	PCR 反应液中漏加组分	确保加了所有的组分。核对 PCR 反应的阳性对照和阴性对照。