武汉昌美生物
Wuhan Charm Biotech

Just-a-Tube™ Genomic DNA Purification from Saliva

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

Product Contents and Storage

The components included with the Just-a-Tube gDNA Purification from saliva are listed below. Upon receipt store all components at room temperature with the exception Proteinase K and RNase A, which need to be stored at 4 ºC. Sufficient reagents are included to perform 50 or 250 genomic DNA extractions.

Product Cat. #	**QT-275-M**	**QT-275-L**
Purification Scale	50	250
Binding Tube (BT3)	50	250
Lysis Buffer (PKLB)	5.5 ml	27.5 ml
Binding Buffer (KB6)	10.0 ml	50.0 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	18.0 ml	90.0 ml
Elution Buffer (EB2)	5.5 ml	27.5 ml
Proteinase K (PK2) (20 mg/ml)	1.1 ml	5.5 ml
RNase A (RA2) (20 mg/ml)	1.1 ml	5.5 ml

Product Description

The Just-a-Tube™ Genomic DNA Purification from Saliva is designed and optimized for easy and fast isolation of high-quality gDNA from fresh saliva, frozen saliva or saliva sample stored in the Charm-Protect Saliva Collection and Storage Vial or preservation solutions from other companies. DNA sample purification can be processed just in a microtube. No silica membrane and magnetic beads are needed for DNA binding in the process. Based on Charm Biotech developed Solid Surface Reversible Binding (SSRB) technology, the Just-a-Tube system utilizes micro-tubes coated with proprietary turbo-binders acting to selectively capture and efficiently bind genomic DNA from saliva cell lysates. In the presence of Binding Buffer gDNA specifically interacts with the turbo binders and binds to the tubes while proteins and other contaminants will remain in the solution. Unbound material is removed in washing steps. The purified gDNA can easily 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, sequencing, whole genome amplification (WGA) and DNA methylation analysis.

Additional Materials Needed

- 100 % ethanol , Isopropanol
- 1.5 ml or 2.0 ml microtubes
- Bench-top centrifuge capable of spinning 1.5-2.0 ml microtubes
- Vortex or Shaker
- Incubator (air or water) or hot blocker

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 QT-275-M kit, add 22 ml of 100 % ethanol to Washing Buffer I (WB1) and mix well. For QT-275-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 QT-275-M kit, add 72 ml of 100 % ethanol to Washing Buffer II (WB2) and mix well. For QT-275-L kit, add 360 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

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").

Experimental Procedure for Charm Protected saliva samples:

1. Add 20 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 or tube sit for 3-5 minutes.
3. Transfer 1 ml saliva preservation mixture 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 20 µl Proteinase K (PK2) into the tube and mix well by inverting the tube 15-18 times.
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)
6. Centrifuge the microtube at maximum speed (about 13000 rpm or 16000 g) for 4 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 20 µl RNase A (RA2) into a 1.5 ml or 2.0 ml microtube.
2. Add 100 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 900 µl saliva or saliva mixture into the microtube preloaded with RNase A (RA2) and PKLB, mix well by pipetting the solution up and down 8 - 9 times or by inverting the microtube 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 20 µl Proteinase K (PK2) into the tube and mix well by inverting the tube 15-18 times.
6. 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)
7. Centrifuge the microtube at maximum speed (about 13000 rpm or 16000 g) for 4 minutes to remove any particulate materials, such as food particles, undigested dead cells and fibers debris.

Binding Products

1. Transfer 900 µl clarified saliva lysate into a Binding Tube (BT3) without collecting the debris pellet at the bottom of the microtube (Note: To avoid the debris pellet, you may leave about 150-250 µl solution in the microtube when doing aspiration).
2. Add 900 µl working Binding Buffer (KB6) containing Isopropanol to the Binding Tube (BT3). Mix well with the saliva lysate by pipetting up and down the solution 10 times or by inverting the microtube 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 (BT3) at maximum speed (about 13000 rpm or 16000 g) at room temperature for 4 minutes to bind the DNA. (Note: During centrifugation, always position microtube hinge pointed outward from the center of rotation. Majority of DNA will collect at the bottom along the hinge side of the mirotube.)

4. Open the cap. Remove the liquid from the Binding Tube. Choose one of the methods listed below to remove the liquid. (1) Decant the solution by flipping the Binding Tube over a waste container and shaking briskly. (2) Remove the solution by aspirating the solution from the center of the bottom with a pipet tip. Be sure not to scrape the walls of the tube with pipet tips during aspiration as the products are bound to the walls.

Washing Products

1. Add 500 µl Washing Buffer I (WB1) containing ethanol to each tube. Mix by shaking the tube 4-5 times. Incubate at room temperature for 1 minute.
2. Centrifuge the Binding Tube at maximum speed (≥ 13000 rpm or 16000 g) at room temperature for one minute. (Note: During centrifugation, always position microtube hinge pointed outward from the center of rotation).
3. Remove the Washing Buffer (WB1) from the Binding Tube (BT3) using one of the methods suggested in step 4 of "Binding Products".
4. Add 800 µl Washing Buffer II (WB2) containing ethanol to the Binding Tube (BT3). Mix by shaking the tube 4-5 times or by inverting the microtube 7-8 times after closing the cap.
5. Centrifuge the Binding Tube at maximum speed (≥ 13000 rpm or 16000 g) at room temperature for one minute. (Note: During centrifugation, always position micro-tube hinge pointed outward from the center of rotation).
6. Remove the Washing Buffer (WB2) from the Binding Tube (BT3) using one of the methods suggested in step 4 of "Binding Products".
7. Repeat steps 4 to 6 above once for a total of two washes with Washing Buffer (WB2).
8. After the final wash, to ensure complete removal of Washing Buffer, you may spin the Binding Tube (BT3) very briefly after removing the majority of solution and aspirate the last drop of liquid at the bottom center of the tube with a 200 µl pipet tip, and air-dry the Binding Tube (BT3) in a lab hood for 8-10 minutes to remove any residual liquid. Alternatively, put the tube in a 62 º C air incubator or a hot blocker and air-dry the tube without cover for 3-5 minutes.

Eluting Genomic DNA

Genomic DNA attached to the walls of the Binding Tube can be stored directly in the well for at least 6 months. If the gDNA is to be eluted, follow the procedure below

1. Add 40-150 µl Elution Buffer (EB2) into Binding Tube. (If higher concentration is preferred, add 40 µl Elution Buffer (EB2) incubate at 62 ºC for 5-10 minutes.
2. Vortex the tube for 30-60 seconds or shake the tube for 5 minutes. If the liquid is cloudy or has any particulate materials inside, centrifuge the tube at maximum speed for one minute to transfer upper clear liquid to a new clean tube.
3. The eluted gDNA may be used immediately in downstream applications. Alternatively, the eluted gDNA may be stored in the Binding 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. A260/A280 values are in the range of 1.6-1.9 (which are lower than standard values, but there is no any problem for any downstream molecular assays based on our data showed). The yield is about 4-25 µg. 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 in different individual saliva samples	Increase the starting saliva sample volume for DNA isolation. Please call us for exact procedure if you want to process whole 4 ml saliva mixtures.
No PCR product	Missing Component in the PCR mixture	Be sure to add all components. Check positive control and negative control for PCR reaction.

Just-a-Tube™ 唾液 gDNA 提纯试剂盒

室温保存，Proteinase K 和 RNase A 保存在 4 ºC。

试剂盒组成及贮存

Just-a-Tube™ 唾液 gDNA 提纯试剂盒可从 1 ml 唾液中提纯高质量的 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 次唾液 gDNA 的提纯。

Product Cat. #	**QT-275-M**	**QT-275-L**
Purification Scale	50 次	250 次
Binding Tube (BT3)	50 个	250 个
Lysis Buffer (PKLB)	5.5 ml	27.5 ml
Binding Buffer (KB6)	10.0 ml	50.0 ml
Washing Buffer I (WB1)	5.5 ml	27.5 ml
Washing Buffer II (WB2)	18.0 ml	90.0 ml
Elution Buffer (EB2)	5.5 ml	27.5 ml
Proteinase K (PK2) (20 mg/ml)	1.1 ml	5.5 ml
RNase A (RA2) (20 mg/ml)	1.1 ml	5.5 ml

产品介绍

Just-a-Tube™ 唾液 DNA 提纯试剂盒可以简单、迅速地从新鲜唾液、冻存唾液、Charm-Protect™ 唾液收集保存瓶的唾液保存液或其他公司唾液保存液中分离提纯高质量的唾液 gDNA。DNA 样品的纯化仅在一个小管中进行。在这个过程中不需要硅膜或磁珠来结合 DNA。昌美生物科技有限公司将固体表面可逆结合技术（SSRB）应用到 Just-a-Tube 系统中，离心管底侧涂有可与唾液裂解液中 DNA 选择性高效结合的结合剂。在结合缓冲液作用下，结合剂可特异性结合 gDNA，而蛋白和其它的成分保留在溶液中。未结合的物质经涮洗后予以去除。纯化的 gDNA 经洗脱液（EB2）或去离子水洗脱。纯化的 gDNA 可直接用于下游反应，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

* 可离 1.5 ml 或 2.0 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。
3. 提纯核酸时，使用无菌新吸头避免污染。

实验前准备

1. 从唾液裂解液中提纯 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。需要制备的 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。
2. QT-275-M 试剂盒，加 22 ml 无水乙醇到 Washing Buffer I（WB1）中，充分混匀。QT-275-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。
3. QT-275-M 试剂盒，加 72 ml 无水乙醇到 Washing Buffer II（WB2）中，充分混匀。QT-275-L 试剂盒，加 360 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。
4. 在进行 gDNA 提纯实验之前，为了避免 gDNA 降解，请遵守标准的 Charm-Protect 唾液 DNA 收集和保存程序。

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”。）

昌美生物 Charm-Protect ™ 唾液保存液保存的唾液操作步骤：

1. 取 20 µl RNase A（RA2）到一个 1.5 ml 或 2.0 ml 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上下颠倒 7-8 次后静置 3-5 分钟。
3. 从唾液收集瓶中吸取 1 ml 唾液与保存液的混合物到预先加了 RNase A（RA2）的离心管中，吸头吹打 8-9 次，或盖上离心管盖子后上下颠倒 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，如食物颗粒。）
4. 取 20 µl Proteinase K（PK2）到 1 ml 唾液混合物中，将离心管盖子盖上后颠倒离心管 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，期间颠倒离心管，也可将样品 52 ºC 温育过夜。
6. 将离心管以最大转速（13000 rpm 或 16000 *g*）离心 4 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 20 µl RNase A（RA2）到一个 1.5 ml 或 2.0 ml 离心管中。
2. 向管中加入 100 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 0.9 ml 唾液或者唾液混合物，加到预先加了 RNase A（RA2）和 PKLB 的离心管中，吸头吹打 8-9 次，或将离心管盖子盖上后颠倒 9-10 次，充分混合均匀。（当吸取唾液混合物时，注意不要吸到底部颗粒状沉淀杂物。）
5. 向管中加 20 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 水浴锅或金属浴中温育 45 分钟，进行 PK2 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. 将离心管以最大转速（13000 rpm 或 16000 *g*）离心 4 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

1. 转移 900 µl 澄清的唾液裂解液到一个结合管（BT3）中，注意不要吸到离心管底的碎片沉淀。注意：为避免吸到管底的碎片沉淀，吸的时候可以留 150-250 µl 溶液在离心管中。
2. 加 900 µl 含有异丙醇的 Binding Buffer（KB6）工作液到结合管（BT3）中，盖上结合管（BT3）盖子，将其颠倒 15-18 次，使 Binding Buffer（KB6）工作液与唾液裂解液充分混匀（这一步非常重要）。
3. 室温下以最大转速（13000 rpm 或 16000 *g*）将结合管（BT3）离心 4 分钟来结合 DNA。注意：在离心过程中，离心管盖朝向转子方向放置，离心后大部分 DNA 聚集在背对管盖的管底侧。
4. 打开盖子，用吸头吸走结合管（BT3）底的液体（注意在吸的过程中，吸头不要碰到管壁，因为 DNA 吸附在管壁上），或者直接将结合管（BT3）中的液体倾倒入废液收集杯中。

清洗 DNA

1. 向结合管（BT3）中加 500 µl 含乙醇的 Washing Buffer I（WB1），盖上盖子，上下颠倒 7-8 次。
2. 室温下以最大转速（≥13000 rpm 或 16000 *g*）离心结合管（BT3）1 分钟（在离心过程中，离心管盖朝向转子方向放置。）。
3. 用吸头吸走结合管（BT3）中的 Washing Buffer I（WB1）（注意在吸的过程中，吸头不要碰到管壁，因为 DNA 吸附在管壁上），或者直接将结合管（BT3）中的液体倒入废液收集杯中。
4. 向结合管（BT3）中加 800 µl 含乙醇的 Washing Buffer II（WB2），盖上盖子，上下颠倒 7-8 次。
5. 在室温以最大转速（≥13000 rpm 或 16000 *g*）离心结合管（BT3）1 分钟。在离心过程中，离心管盖朝向转子方向放置。
6. 用吸头去除结合管（BT3）中 Washing Buffer II（WB2），或者直接将结合管（BT3）中的液体倾倒入废液收集杯中。
7. 重复步骤 4-6，即用 Washing Buffer II（WB2）洗两次。
8. 洗完最后一遍之后，为彻底清除 Washing Buffer，在移除大部分液体后，可再次短暂离心结合管（BT3）30 秒，用 200 µl 吸头吸走管底中间最后一滴液体，将结合管（BT3）在实验室通风橱晾 8-10 分钟来去除残留的液体，也可以将管子置于 62 °C 水浴锅或金属浴中晾 3-5 分钟。

gDNA 的洗脱

吸附在结合管（BT3）管壁的 gDNA 至少可以保存六个月。如果 gDNA 需要洗脱，可进行下面的程序。

1. 加 40-150 µl 洗脱缓冲液（EB2）到结合管（BT3）管壁（如果需要高浓度的 DNA，加 40 µl 洗脱缓冲液（EB2），62 ºC 温育 5-10 分钟，选择方法 1 或方法 2 来洗脱 gDNA。）。
2. 方法 1（涡旋洗脱）: 盖上结合管的盖子，涡旋 30 秒后以最大转速短暂离心，使所有的液体集中到管底。

   方法 2（移液枪洗脱）：用移液枪上下吹打溶液 8-10 次，盖上离心管的盖子，将管子在室温放置 2 分钟。
3. 洗脱的 gDNA（若较浑浊或有颗粒物质，可以最大转速离心 1 分钟，取上清）直接应用于下游反应，或置于 4 ºC 短期储存或者-20 ºC 长期储存。

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量产量。A260/A280 比值在 1.6－1.9 之间（比常规的值偏低，但不影响任何下游分子生物学实验）。结合管（BT3）中纯化的 gDNA 可直接用于下游反应，如限制性酶切反应、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。	确保遵守正确手册推荐的样品收集和保存程序。
	裂解不彻底。	62 ºC 温育时间延长 15-30 分钟。
	样品本身量不同（统计发现，每个人唾液中 DNA 含量是不同的，小孩 DNA 量少，老年人 DNA 量多）。	增加要提纯的唾液使用量，用 2 ml - 4 ml 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。