

## BLOOD & TISSUE GENOMIC DNA EXTRACTION KIT

DE 047/ DE 049

Whole Blood, Tissues (Fresh/Frozen-Fixed: Paraffin, Formalin), Bacteria, Yeast, dried blood spots, water samples, Amniotic fluid, Sea Urchins, marine mollusks, octopus and insects.

### Description

The Blood & Tissue Genomic DNA Extraction Kit is designed for rapid extraction of pure, small-scale genomic & mitochondrial DNA from several types of tissues (fresh or frozen), fixed tissues (Formalin, Paraffin), Whole Blood, buffy coat, bacteria, yeast, fungi and, saliva, blood spot, viral, hair, bone tissue, dental tissue, insects, amniotic fluid, sea urchins, marine mollusks, octopus and insects.

### Highlights:

- Rapid isolation cellular DNA from animal tissue (e.g. mouse tails), bacteria, yeast etc.
- Purified DNA is ready for downstream application such as PCR, Southern blotting.
- Centrifugation-based method.
- Efficiently remove cellular debris and inhibitors
- No phenol/chloroform extraction and ethanol precipitation.

### Applications

PCR  
Southern Blotting  
Medicolegal Analysis

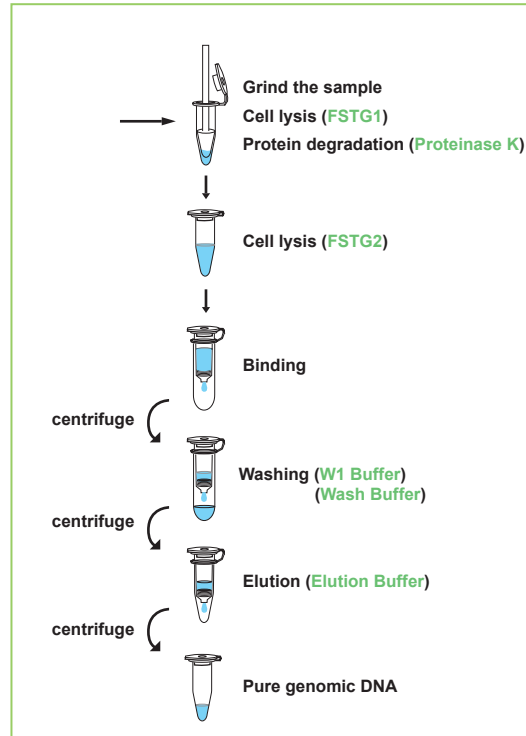
**Principle:** mini spin column (silica matrix) 30 ~  
**Operation time:** 60 minutes  
**Binding capacity:** up to 60 ug DNA/ column  
**Typical yield:** 15 ~35 ug/ prep - Blood 4-8 ug/200ul

**Column applicability:** centrifugation and vacuum  
**Minimum elution volume:** 50 ul

### Sample size:

**Blood:** up to 200 ul whole blood, serum, plasma, body fluids  
up to  $5 \times 10^6$  cultured cells  
**Tissues:** < 25 mg animal tissue

1.2 cm mouse tail



### Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH<sub>2</sub>O to Proteinase K tube to make a **10 mg/ml** stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add ethanol (96- 100 %) to **W1 Buffer** and **Wash Buffer** when first open.
4. Prepare dry baths or water baths before the operation: one to 60 °C for step 4 and the other to 70 °C
5. Preheat the Elution Buffer to 70 °C for step 13.
6. All centrifuge steps are done at full speed (~ 18,000 x g) in a microcentrifuge.

| Cat. No:                                | DE-047s<br>(4preps_sample) | DE-047<br>(100 preps) | DE-049<br>(300 preps) |
|---|----------------------------|-----------------------|-----------------------|
| FSTG1 Buffer                            | 1.5 ml                     | 30 ml                 | 70 ml                 |
| FSTG2 Buffer                            | 1.5 ml                     | 30 ml                 | 70 ml                 |
| Proteinase K (lyophilized) <sup>o</sup> | 1 mg                       | 11 mg x 2             | 11 mg x 6             |
| W 1 Buffer * (concentrate)              | 1.3 ml                     | 44 ml                 | 124 ml                |
| Wash Buffer **                          | 1 ml                       | 20 ml                 | 50 ml                 |
| (concentrate) Elution                   | 1 ml                       | 30 ml                 | 90 ml                 |
| Buffer FSTG Mini Column                 | 4 pcs                      | 100 pcs               | 300 pcs               |
| Collection Tube                         | 8 pcs                      | 200 pcs               | 600 pcs               |
| Elution Tube Micropestle                | 4 pcs                      | 100 pcs               | 300 pcs               |
| User Manual                             | 4 pcs                      | 100 pcs               | 300 pcs               |
|   | 1                          | 1                     | 1                     |

|   |        |        |
|---|--------|--------|
| Preparation of Proteinase K solution (10 mg/ml)<br>by adding ddH <sub>2</sub> O |        |        |
| *ddH <sub>2</sub> O volume for Proteinase K <sup>o</sup>                        | 0.1 ml | 1.1 ml |

|  |        |       |        |
|--|--------|-------|--------|
| Preparation of W1 Buffer and Wash buffer by adding ethanol (96-100%) |        |       |        |
| *Ethanol volume for W1 Buffer  | 0.5 ml | 16 ml | 45 ml  |
| ** Ethanol volume for Wash Buffer                                    | 4 ml   | 80 ml | 200 ml |

## Tissue ; eberal Protocol (Fresh/Frozen):

Please Read Important Notes Before Starting The Following Steps.

For other special samples, please refer to special Protocol and choose the appropriate one.

**Additional requirements:** RNase A (optional), 96-100% ethanol

**Hint:** Prepare two dry baths or two water baths before the operation: One to 60°C for step 4 and other to 70°C for step 7.

|                                     |  |
|-------------------------------------|--|
| <p>Step<br/>Tissue Dissociation</p> | <p>1. <b>(For fresh sample)</b> Cut up to 25 mg tissue sample to a microcentrifuge tube (not provided). Use provided Micropestle to grind the tissue sample and break it into small pieces. Or you can grind the tissue sample in liquid nitrogen then transfer the powder to a new microcentrifuge tube.<br/> <b>(For frozen sample)</b> Weigh up to 25 mg tissue sample and grind the tissue sample in liquid nitrogen then transfer the powder to a microcentrifuge tube.</p> <ul style="list-style-type: none"> <li>• If tissue sample has a high number of cells (e.g. Spleen), no more 10 mg should be used.</li> </ul> <p>2. Add 200 µl FSTG1 Buffer and homogenize the tissue sample more completely with micropestle.</p>   |
| <p>Step<br/>Lysis</p>               | <p>3. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by vortexing.<br/> 4. <b>Incubate at 60°C until the tissue is lysed completely (1-3h)</b>. Vortex occasionally during incubation.<br/> --- Sample can be incubated overnight as well as for complete lysis.<br/> 5. Briefly spin the tube to remove drops from the inside of the lid.<br/> 6. <b>(Optional)</b> If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml) (not provided)incubate for 2 min at room temperature.<br/> 7. Add 200µl <b>FSTG2 Buffer</b> to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70° C for 10 min.<br/> 8. Briefly spin the tube to remove drops from the inside of the lid.<br/> If there is insoluble material present, centrifuge at full speed for 2 min and transfer the supernatant to a new microcentrifuge tube. (Not provided)</p> |
| <p>Step<br/>DNA Binding</p>         | <p>9. Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse-vortexing.<br/> 10. Briefly spin the tube to remove drops from the inside of the lid.<br/> 11. Place a FSTG Mini Column in a Collection Tube. Transfer the sample any precipitate) carefully to FSTG Column. Centrifuge for 1 min and discard the flowthrough then place FSTG Column to a new Collection Tube.</p>   |
| <p>Step<br/>Wash</p>                | <p>12. Add 400µl W1 Buffer to FSTG Columnby centrifuge for 1 min then discard the flow-through.<br/> <ul style="list-style-type: none"> <li>• Make sure that ethanol has been added into W1 Buffer when first open.</li> </ul> 13. Add 750µl Wash Buffer to FSTG Column by centrifuge for 1 min then discard the flow-through.<br/> 14. Centrifuge for an additional 3 min to dry the column.<br/> <ul style="list-style-type: none"> <li>• Make sure that ethanol has been added into Wash Buffer when first open.</li> <li>• <b>Important Step!</b> This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.</li> </ul> </p>  |
| <p>Step<br/>DNA Elution</p>         | <p>15.Add 100µl of pre-heated Elution Buffer or ddH2O(pH 7.5 -8.5)to the membrane center of FSTG Column. Stand FSTG Column for 3 min.<br/> <ul style="list-style-type: none"> <li>• <b>Important Step!</b> For effective elution, make surethat the elution solution is dispen on the membrane center and is absorbed completely.</li> <li>• Standard volume for elution is 200µl. If tissue sample has a low number of cells, reduce the elution volume(50-150 µl) to increase DNA concentration.</li> </ul> 16. Centrifuge at full speed for 2 min to elute total DNA.<br/> 17. Store total DNA at 4°C or -20°C.</p>   |

## **: il eX Tissue fParaZb/ : ora alibŁ**

Additional requirements: RNase A (optional), 96-100% ethanol  
Xylene for paraffin embedded tissues -  
Set 2 dry or water baths one to 60°C and the other to 70°C

### • For paraffin-embedded tissues

1. Cut up to 25 mg paraffin-embedded tissue sample to a microcentrifuge tube (not provided).
2. Add 1 ml xylene, mix well and incubate at room temperature for 30 min.
3. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
4. Add 1 ml ethanol (96 ~ 100%) to the deparaffined tissue, mix gently by vortexing.
5. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
6. Add 1 ml ethanol (96 ~ 100%) to the deparaffined tissue, mix gently by vortexing.
7. Centrifuge at full speed for 5 min. Remove the supernatant by pipetting.
8. Incubate at 37° C for 10 min to evaporate ethanol residue.
9. Grind the tissue sample by micropestle or liquid nitrogen.
10. Add 200µl FSTG1 Buffer to the sample mixture, mix well by Micropestle with pipette tips
11. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by vortexing
12. Incubate at 60° C until the tissue is lysed completely (usually in 1 hr- 3 hr, depends on the Vortex every 10 minutes during incubation.  
--Sample can be incubated overnight as well for complete lysis.
13. **(Optional)** If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml) not provided . Mix thoroughly by vortexing and incubate for 2 min at room temperature.
14. Briefly spin the tube to remove drops from the inside of the lid.  
If there is insoluble material present, centrifuge at full speed for 2 min and transfer the supernatant to a new microcentrifuge tube.
15. Add 200µl FSTG2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70° C for 10 min

Step  
Lysis

16. Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse-vortexing.
17. Briefly spin the tube to remove drops from the inside of the lid.
18. Place a FSTG Mini Column in a Collection Tube. Transfer the sample (any precipitate) carefully to FSTG Column. Centrifuge for 1 min at full speed , discard the flowthrough, then place FSTG Column to a new Collection Tube.

Step  
DNA Binding

19. Add 400 ul W1 Buffer to the FATG Mini Column ,centrifuge at full speed and discard the flowthrough.

Step  
Wash

- Make sure that ethanol has been added into W1 Buffer when first open.
20. Add 750 ul Wash Buffer to the FSTG Column by centrifuge for 1 min then discard the flow-through.  
--Make sure that ethanol has been added into Wash Buffer when first open.
  21. Centrifuge for an additional 3 min to dry the column.
    - **Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Step  
DNA Elution

22. Add 100 ul of preheated Elution Buffer or ddH<sub>2</sub>O (pH 7.5 ~ 9.0)to the membrane center of of FSTG Mini Column. Stand FSTG Mini Column for 3 min.
  - **Important Step!** For effective elution, make surethat the elution solution is dispensed on the membrane center and is absorbed completely.
  - Standard volume for elution is 100µl.If tissue sample has a low number of cells, reduce the elution volume to 50 µl to increase DNA concentration.
23. Centrifuge for 2 min to elute total DNA.
24. Store total DNA at 4°C or -20°C.

## : il eX Tissues ft ora alibŁ

1. Wash 25mg tissue sample twice with 1 ml PBS to remove formalin.
2. Grind the tissue sample by micropestle or liquid nitrogen .
3. Add 200µl FSTG1 Buffer to the sample mixture, mix well by Micropestle with pipette tips
4. Add 20µl Proteinase K(10mg/ml) to the sample mixture. Mix thoroughly by
5. Incubate at 60 °C until the tissue is lysed completely (usually in 1 hr, depends on the sample types). Vortex every 10-15 min during incubation.

Step  
Lysis

6. Briefly spin the tube to remove drops from the inside of the lid.
7. (Optional) If RNA free genomic DNA is required, add 4µl of RNase A (100 mg/ml)
8. Add 200µl FSTG Buffer to the sample mixture, mix well by Micropestle with pipette tips and incubate at 70° C for 10 min.
9. Briefly spin the tube to remove drops from the inside of the lid.
  - If there is insoluble material present, centrifuge at full speed for 2 min and transfer the supernatant to a new microcentrifuge tube. (Not provided)

Step  
DNA Binding

- Add 200 µl ethanol (96 ~ 100%) to the sample. Mix thoroughly by pulse
  - Briefly spin the tube to remove drops from the inside of the lid.
  - Place a FSTG Mini Column in a Collection Tube. Transfer the sample (any precipitate) carefully to FSTG Column. Centrifuge for 1 min at full speed , discard the flowthrough, then place FSTG Column to a new Collection Tube.
- 1 . Add 400 ul W1 Buffer to the FATG Mini Column ,centrifuge at full speed and discard the flowthrough.

Step  
Wash

- Make sure that ethanol has been added into W1 Buffer when first open.
  - Add 750 ul Wash Buffer to the FSTG Column by centrifuge for 1 min then discard the flow-through.
  - --Make sure that ethanol has been added into Wash Buffer when first open.
- 1 • Centrifuge for an additional 3 min to dry the column.
    - **Important Step!** This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

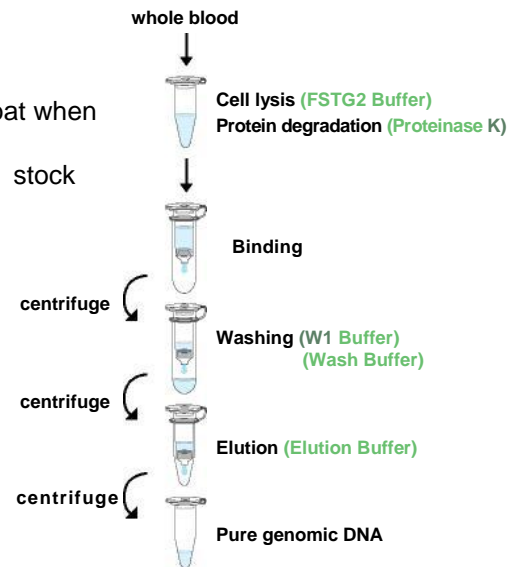
Step  
DNA Elution

- Add 100 ul of preheated Elution Buffer or ddH<sub>2</sub>O (pH 7.5 ~ 9.0)to the membrane center of FSTG Mini Column. Stand FSTG Mini Column for 3 min.
- **Important Step!** For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely.
- Standard volume for elution is 100µl. If tissue sample has a low number of cells, reduce the elution volume to 50 µl to increase DNA concentration.
- Centrifuge for 2 min to elute total DNA.
- Store total DNA at 4°C or -20°C.

## Special protocol for extraction of Genomic DNA and Viral DNA from blood

### Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH<sub>2</sub>O to Proteinase K tube to make a 10 mg/ml stock solution.  
Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first open.
4. Preheat a dry bath or water bath to 60 °C before the operation.
5. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.



### General Protocol for whole blood:

#### Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube (not provided). - If the sample volume is less than 200 µl, add the appropriate volume of PBS.
2. **(Optional):** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided) to the sample and incubate for 2 min at room temperature.
3. Add 20 µl of Proteinase K and 200 µl of FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** - Do not add Proteinase K directly to FSTG2 Buffer.
4. Incubate at 70 °C for 10 minutes to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
5. Briefly spin the tube to remove drops from the inside of the lid.
6. Add 200 µl of ethanol (96- 100 %) to the sample. **Mix thoroughly by pulse-vortexing for 30 sec.**
7. Briefly spin the tube to remove drops from the inside of the lid.
8. Place a FSTG column to a Collection Tube.  
Transfer the mixture carefully to FSTG Column. Centrifuge for 1 minute **then place FSTG Column to a new Collection Tube.**
9. Add 400 µl W1 Buffer to the FSTG Column by centrifuge for 1 minute then discard the flow-through.
10. Add 750 µl Wash Buffer to the FSTG Column with by centrifuge for 1 min then discard the flow-through. - Make sure that ethanol has been added into Wash Buffer when first open.
11. **Centrifuge for an additional 3 minutes to dry the column.**  
**Important Step!** The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.
12. Place the FSTG Column to a Elution Tube.
13. Add 100 ~ 200 µl of Elution Buffer or ddH<sub>2</sub>O (pH 7.0) to the membrane of the FSTG Column.  
**Stand the FSTG Column for 3 minutes.**  
- **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
14. Centrifuge at 14,000 x g for 1 minute to elute total DNA.
15. Store total DNA at 4 °C or -20 °C.

### **Protocol: Isolation of DNA from Animal Cultured Cells**

Additional requirement: RNase A (optional), 96~100% ethanol, trypsin or cell scraper (for monolayer cell), PBS Hint: Set dry or water baths: 60 °C and 70 °C

1. Harvest cells

a. Cells grown in suspension

I. Transfer the appropriate number of cell ( up to  $5 \times 10^6$  ) to a microcentrifuge tube.

II. Centrifuge at 300 x g for 5 min.

III. Discard supernatant carefully and completely.

b. Cells grown in monolayer

I. Detach cells from the dish or flask by trypsinization or using a cell scraper.

II. Transfer the appropriate number of cell ( up to  $5 \times 10^6$  ) to a microcentrifuge tub

III. Centrifuge at 300 x g for 5 min. Discard supernatant carefully and completely.

IV. Discard supernatant carefully and completely.

2. Resuspend cell pellet in PBS to a final volume of 200 ul.

3. Follow the Animal Tissue Protocol starting from step 2.

### **Preparation of buffy coat:**

Centrifuge whole blood at 3,300 g for 10 min at room temperature and you will get three different fractions: the upper clear layer is plasma: the intermediate layer is buffy coat, containing concentrated leukocytes: the bottom layer contains concentrated erythrocytes.

Process the General Protocol from Step.1 for buffy coat .

Extraction total DNA from buffy coat will yield 5-10 times more DNA than an equivalent volume of whole blood.



## Bacteria Protocol

Additional equipment: RNase A (optional) -Ethanol 96-100%

For Gram-Positive bacteria: lysozyme reaction solution (20 mg/ml lysozyme: 20mM TrisHCL, pH8.0,2mM EDTA 1.2% Triton)

Set: dry or water baths: 60°C ,another for step 4 and 70°C for step 6

- **For bacterial cultures**

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 min and discard the supernatant completely.
3. Follow the General Protocol starting from step 2.

- **II. For bacteria in biological fluids**

1. Collect bacteria by centrifuging biological fluids at 7,500 rpm (5,000 xg) for 10 min.
2. Follow the General Protocol starting from step 2.

- **III. For bacteria from eye, nasal, pharyngeal, or other swabs**

1. Soak the swabs in 2 ml PBS at room temperature for 2-3 hr.
2. Collect bacteria by centrifuging at 7,500 rpm (5,000 xg) for 10 min.
3. Follow the General Protocol starting from step 2.

- **IV. For Gram-positive bacteria**

**Hint:** Preheat two dry baths or two water baths before the operation: one to 60°C and the other to 95°C for step 7.

1. Transfer 1 ml well-grown bacterial culture to a microcentrifuge tube (not provided).
2. Descend the bacterial cells by centrifuging at full speed for 2 min and discard the supernatant completely.
3. Resuspend the cell pellet in 200 µl lysozyme reaction solution (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton).
4. Incubate at 37 °C for 30 min.
5. **(Optional):** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A and incubate for 2 min at room temperature.
6. Add 20 µl Proteinase K to the sample, and then add 200 µl FSTG2 Buffer to the sample. Mix thoroughly by pulse-vortexing.
7. Incubate at 60 °C for 30 min and then for a further 15 min at 95°C .
8. Follow the General Protocol starting from step 9.

## For Yeast

Additional equipment: RNase A (optional) -Ethanol 96-100%

Zymolyase or Lyticase , 200U for one preparation

Sorbitol buffer (1M sorbitol: 100 mM EDTA, 14 mM Beta-mercaptoethanol)

Set: dry or water baths: one to 30°C ,another 60°C and the other to 70°C

1. Transfer 3 ml log-phase (OD600 =10) yeast culture to a microcentrifuge tube (not provided).
2. Descend the yeast cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard the supernatant completely.
3. Resuspend the cell pellet in 600 µl sorbitol buffer ( 1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol). Add 200 Zymolyase or lyticase and incubate at 30°C for 30 min.
4. Centrifuge at 7,500 rpm (5,000 x g) for 5 min. Remove the supernatant by pipetting.
5. Follow the General Protocol starting from step 2.

## For Dried Blood Spot

Additional equipment: RNase A (optional) -Ethanol 96-100%

**Hint:** Preheat three dry baths or three water baths before the operation: one to 85 °C for step 2, another to 60 °C for step 5 and the other to 70 °

1. Cut the filter paper (e.g. S&S 903) with dried blood spot into a microcentrifuge tube (not provided).
2. Add 200µl FSTG1 Buffer and incubate at 85 °C for 10 min.
3. Briefly spin the tube to remove drops from the inside of the lid.
4. Add 20µl Proteinase K to the sample mixture. Mix thoroughly by vortexing.
5. Incubate at 60 °C for 1 hr. Vortex every 10-15 min during incubation.
6. Follow the General Protocol starting from step 9.

## Special protocol for extraction of Gram-positive bacterial DNA from Saliva

The additional requirement:

1. PBS
2. Lysozyme reaction solution: (20 mg/ml lysozyme; 20 mM Tris-HCl, pH 8.0; 2mM EDTA; 1.2 % Triton X-100) (200ul/preparation)
3. Incubator at 37 °C for step 4
4. Two incubators for step 6: one to 60 °C and the other to 95 °C
5. RNase A (50mg/ml) for Optional Step (step 7)
6. Absolute ethanol (96~100 %)

### Protocol

1. Transfer 1 ml saliva sample to a 15 ml centrifuge tube. Add 4 ml PBS (not provided) and mix well.
2. Centrifuge the tube at 1,800 x g for 5 min.
3. Carefully discard the supernatant. Resuspend the pellet in 200 ul lysozyme reaction solution and transfer the sample mixture to a 1.5 ml microcentrifuge tube.
4. Incubate the sample mixture at 37 °C for 30 min.
5. Add 20 ul Proteinase K to the sample mixture and add 200 ul FSTG2 Buffer to the sample mixture. Mix thoroughly by pulse-vortexing.
6. Incubate at 60 °C for 30 min and then for a further 15 min at 95 °C.
7. (Optional): If RNA-free genomic DNA is required, add 6 ul of 50 mg/ml RNase A to the sample mixture and incubate at room temperature for 2 min .
8. Add 200 ul ethanol (96- 100 %) to the sample mixture. Mix thoroughly by pulse-vortexing for 10 sec.
9. Combine a FSTG Mini Column with a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to the FSTG Mini Column. Centrifuge for 1 minute and place the FSTG Mini Column to a new Collection Tube.
10. Add 500 ul W1 Buffer to the FSTG Mini Column, centrifuge for 1 min, discard the flow-through and place the FSTG Mini Column back to the Collection Tube.
11. Add 750 ul W2 Buffer to the FSTG Mini Column, centrifuge for 1 min, discard the flow-through and place the FSTG Mini Column back to the Collection Tube.
12. Repeat step 11
13. Centrifuge for an additional 3 min to dry the FSTG Mini column.

**Important Step!**

The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.

14. Place the FSTG Mini Column to a Elution Tube.
15. Add 100 ~ 200 ul of Elution Buffer or ddH<sub>2</sub>O (pH 7.5- 9.0) to the membrane center of the FSTG Mini Column. Stand the FSTG Column for 1 minutes.
  - Important Step! For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
  - Standard volume for elution is 200 ul. If less sample to be use, reduce the elution volume (50~150 UI) to increase DNA concentration.
16. Centrifuge for 2 minutes to elute total DNA.

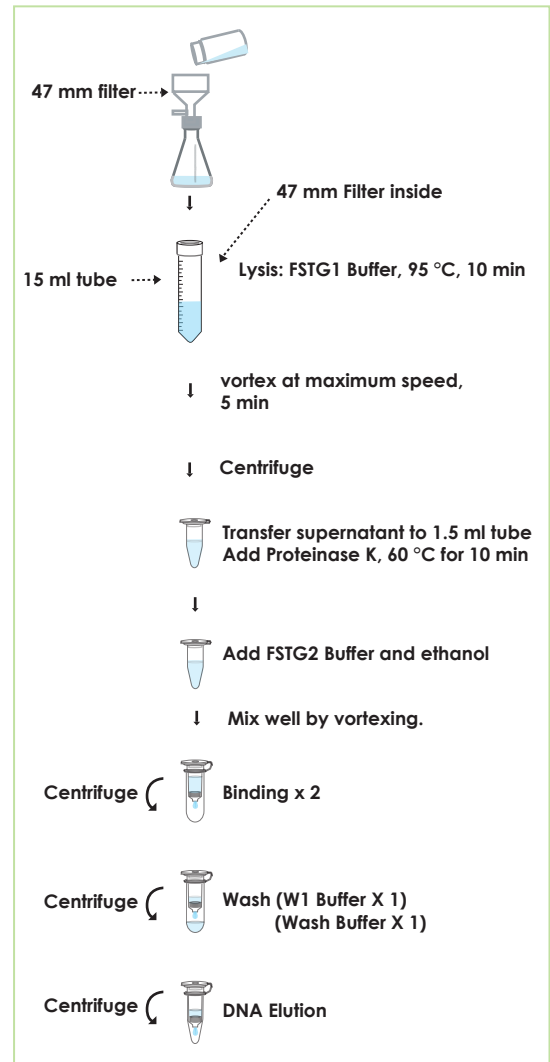


## Special Protocol for Extraction of DNA from Amniotic Fluid:

1. Transfer up to 1 ~ 3 ml amniotic fluid to a centrifuge tube (not provided). Centrifuge at 10,000 x g for 5 min then remove the supernatant.
2. Wash the cell pellet with 1 ml of PBS. Centrifuge at 10,000 x g for 3 min then remove the supernatant completely.
3. Add 200 µl of PBS and resuspend the cells by pipetting. Transfer the sample mixture to a 1.5 ml micro-centrifuge tube. (not provided)
4. **(Optional):** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 min at room temperature.
5. Add 20 µl Proteinase K and 200 µl FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** - Do not add Proteinase K directly to FSTG2 Buffer.
6. Incubate at 60 °C for 15 minutes to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
7. Centrifuge the tube at 10,000 x g for 3 min and transfer the clarified supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
8. Add 200 µl of ethanol (96- 100 %) to the sample mixture. **Mix thoroughly by pulse-vortexing for 30 sec.**
9. Briefly spin the tube to remove drops from the inside of the lid.
10. Place a FSTG Mini Column in a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to the FSTG Mini Column. Centrifuge at 8,000 x g for 30 sec **then place the FSTG Mini Column to a new Collection Tube.**
11. Wash the FSTG Mini Column with 500 µl W1 Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow-through.
12. Wash the FSTG Mini Column with 750 µl Wash Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow -through. - Make sure that ethanol has been added into Wash Buffer when first open.
13. **Centrifuge the FSTG Mini Column at full speed (~18,000 x g) for an additional 3 minutes to dry the column.**
14. Place the FSTG Mini Column to Elution Tube.
15. Add 100 ~ 200 µl of Elution Buffer or ddH<sub>2</sub>O (pH 7.5- 9.0) to the membrane center of FSTG Mini Column. **Stand FSTG Column for 3 minutes.**
  - **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
16. Centrifuge at full speed (~18,000 x g) for 1 min to elute total DNA

## Special protocol for water DNA extraction by using Tissue Genomic DNA Mini Kit

1. Use 47 mm of filter paper with pore size 0.22  $\mu\text{m}$  (or 0.45  $\mu\text{m}$ ) and filter funnel (not provided) to filter the water samples.
2. Pick up the filter from funnel and roll the filter paper into a cylinder with the top side inward.
3. Insert the filter into a 15 ml tube. Add 1000  $\mu\text{l}$  of FSTG1 Buffer. Vortex the tube at maximum speed for 5 min.
4. Incubate the sample at 95  $^{\circ}\text{C}$  for 10 min and vortex the sample twice during the incubation.
5. Centrifuge the tube at 2,500  $\times$  g for 3 min. Transfer 600  $\mu\text{l}$  of the supernatant to a 1.5 ml centrifuge tube. Add 30  $\mu\text{l}$  of Proteinase K and incubate at 60  $^{\circ}\text{C}$  for 10 min.
6. Add 300  $\mu\text{l}$  of FSTG2 Buffer and 450  $\mu\text{l}$  of ethanol. Mix well by vortexing.
7. Place a FSTG Column into a Collection Tube and transfer up to 700  $\mu\text{l}$  of the sample mixture to the Column. Centrifuge at full speed ( $\sim$ 18,000  $\times$  g) for 30 sec then discard the flow-through. Place the column back to the Collection Tube.
8. Repeat the step 7 for the rest of the sample mixture.
9. Add 400  $\mu\text{l}$  of W1 Buffer (ethanol added) to the Column. Centrifuge at full speed for 30 sec then discard the flow-through. Place the column back to the Collection Tube.
10. Add 650  $\mu\text{l}$  of Wash Buffer (ethanol added) to the Column. Centrifuge at full speed for 30 sec then discard the flow-through. Place the column back to the Collection Tube.
11. Centrifuge at full speed ( $\sim$ 18,000  $\times$  g) for an additional 3 min to dry the column.
12. Place the column into a 1.5 ml microcentrifuge tube (not provided). Add 50  $\mu\text{l}$  of preheated Elution Buffer or ddH<sub>2</sub>O to the membrane center of the column. Stand the column for 2 min at room temperature.
13. Centrifuge at full speed for 1 min to elute DNA.



## Protocol: for extraction of DNA from insect

### Important Notes:

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Add 1.1 ml sterile ddH<sub>2</sub>O to Proteinase K tube to make a 10 mg/ml stock solution.  
Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
3. Add required volume of ethanol (96- 100 %) to W1 Buffer and Wash Buffer when first open.
4. Preheat a dry bath or water bath to 60 °C and 70 °C before the operation.
5. All centrifuge steps are done at full speed (14,000 rpm or 18,000 x g) in a microcentrifuge.

### Please Read Important Notes Before Starting The Following Steps.

1. Transfer up to 100 µl of sample that disrupted by a tissue homogenizer to a microcentrifuge tube (not provided).
2. Add 100 µl of Elution Buffer to the sample. Mix well by slapping the tube.
3. Add 200 µl of FSTG1 Buffer and 20 µl of Proteinase K to the sample. **Mix thoroughly by pulse-vortexing.**
4. Incubate at 60 °C for 10 min to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
5. Centrifuge the sample mixture at 18,000 x g for 1 min to descent the debris.
6. Transfer the supernatant to a new 1.5 ml tube.
7. **(Optional):** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A (not provided) to the sample and incubate for 2 min at room temperature.
8. Add 200 µl of FSTG2 Buffer to the sample. **Mix thoroughly by pulse-vortexing.** Incubate at 70 °C for 10 min.
9. Add 200 µl of ethanol (96- 100 %) to the sample. **Mix thoroughly by pulse-vortexing for 30 sec.**
10. Place a FSTG column to a Collection Tube. Transfer the mixture carefully to FSTG Column and centrifuge for 1 minute. **Place FSTG Column to a new Collection Tube.**
11. Wash the FSTG Column with 400 µl W1 Buffer by centrifuge for 1 minute then discard the flow-through.
12. Wash the FSTG Column with 650 µl Wash Buffer by centrifuge for 1 min then discard the flow-through.  
- Make sure that ethanol has been added into Wash Buffer when first open.
13. **Centrifuge for an additional 3 min to dry the column.**  
**Important Step!** The residual liquid can affect the quality of DNA and inhibit subsequent enzymatic reactions.
14. Place the FSTG Column to a Elution Tube.
15. Add 50 ~ 100 µl of Elution Buffer or ddH<sub>2</sub>O (pH 7.0) to the membrane of the FSTG Column.  
**Stand the FSTG Column for 3 min.**  
- **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
16. Centrifuge at 18,000 x g for 1 min to elute DNA.

## PROTOCOL: DNA ISOLATION FROM MARINE MOLLUSKS TISSUES & SEA URCHINS

### PLEASE READ THE **IMPORTANT NOTES** BEFORE STARTING THESE STEPS

#### NOTES:

- Prepare dry baths to 60 °C for step 3, and to 70 °C for step 4.
- Preheat **Elution** Buffer to 70 °C for step 12 (elution step).

#### Sample lysis

1. Cut **up to 25 mg** of mollusk tissue sample to a microcentrifuge tube (not provided).  
Use a micro-pestle (provided) to grind the mollusk tissue.
2. Add 200 µl of **FSTG1** Buffer to the sample. Mix thoroughly by pipetting up and down to disrupt the sample mixture.
3. Add 20 µl of Proteinase K to the sample. Mix thoroughly by vortexing. Incubate the sample mixture at **60 °C** until the sample is completely lysed (invisible) (15 min~ 3 hrs).

**OPTIONAL:** If RNA-free DNA is required, add 8 µl of 100 mg/ml **RNase A** to the sample and incubate for 2 min at room temperature.

4. Add 200 µl of **FSTG2** Buffer to the sample. Mix thoroughly by vortexing.  
Incubate the sample mixture at **70 °C** for 10 min.
5. Cool down the sample at room temperature for 1 min.  
Add 200 µl ethanol (96- 100 %) to the sample. Mix thoroughly by pulse-vortexing for 5 sec.

#### DNA binding

6. **Centrifuge** Place a **FSTG Column** in a Collection Tube,  
**Vacuum** or insert the **FSTG Column** tip into the vacuum adaptor (do not discard the Collection Tube).
7. Transfer the mixture to the **FSTG Column**  
**Centrifuge** Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.  
**Vacuum** Apply a vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from the manifold.

#### Wash the column's membrane

8. Add 500 µl of **W1** Buffer to the **FSTG Column**.  
**Centrifuge** Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.  
**Vacuum** Apply vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from the manifold.
9. Add 700 µl of **Wash** Buffer (ethanol added).  
**Centrifuge** Centrifuge at 18,000 x g for 30 sec. Discard the flow-through and return the **FSTG Column** back to the Collection Tube.  
**Vacuum** Apply vacuum of -6 inches Hg until the column is empty. Switch off the vacuum and release vacuum from the manifold. Place the **FSTG Column** to the Collection Tube.

#### Dry the column's membrane

10. Centrifuge at 18,000 x g for an additional 2 min to completely remove the residual liquid.  
**Important Step!** This step will avoid residual liquids to inhibit subsequent enzymatic reactions.

#### Elution

11. Place the **FSTG Column** to a clean 1.5 ml tube (not provided)
12. Add 40 ~100 µl of **Elution** Buffer to the center of the column. Stand the column for 1 min.  
**Important Step!** For effective elution ensure the solution is dispensed onto the membrane center and is fully absorbed.
13. Centrifuge at 18,000 x g for 30 sec to elute DNA.

## Trouble Shooting

Low or no yield  
of genomic DNA

1. Low amount of cells in the sample
  - Concentrate a larger volume of a new sample to 200  $\mu$ l.
2. Poor Cell Lysis-because of insufficient Proteinase K Activity
  - Repeat the extraction procedure with a new sample. Use a fresh or well-stored Proteinase K stock solution.
- 3.1. Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer
  - Repeat the extraction procedure with a new sample. Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse-vortexing.
- 3.2. Poor Cell Lysis-because of insufficient incubation time
  - Repeat the extraction procedure with a new sample. Extend the incubation time and make sure that no residual particulates remain.
- 3.2. Ethanol is not added into the lysate before transferring into FSTG Mini Column
  - Repeat the extraction procedure with a new sample.
- 4.1. Incorrect preparation of wash buffer-Ethanol is not added into wash buffer when first open
  - Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open. Repeat the extraction procedure with a new sample.
- 4.2. Incorrect preparation of wash buffer-The volume or the percentage of ethanol is not correct before adding into wash buffer
  - Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open. Repeat the extraction procedure with a new sample.
- 5.1. Elution of genomic DNA is not efficient-pH of water (ddH<sub>2</sub>O) for elution is acidic
  - Make sure the pH of ddH<sub>2</sub>O is between 7.5-9.0 or use Elution Buffer (provided) for elution.
- 5.2. Elution of genomic DNA is not efficient-Elution buffer or ddH<sub>2</sub>O is not completely absorbed by column membrane
  - After Elution buffer or ddH<sub>2</sub>O is added, stand the FSTG Mini column for 5 min before centrifugation.

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| <p>Brown residues remain on the column membrane after washing</p> | <p>1.1 Poor Cell Lysis-because of insufficient Proteinase K Activity</p> <ul style="list-style-type: none"> <li>• Repeat the extraction procedure with a new sample. Use a fresh or well store Proteinase K stock solution.</li> <li>• Don't add Proteinase K Directly to FSTG2 buffer.</li> </ul> <p>1.2 Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer</p> <ul style="list-style-type: none"> <li>• Repeat the extraction procedure with a new sample. Mix the sample and Buffer immediately and thoroughly by pulse-vortexing.</li> </ul> <p>1.3 Poor Cell Lysis-because of insufficient incubation time</p> <ul style="list-style-type: none"> <li>• Repeat the extraction procedure with a new sample.</li> <li>• Extend the Incubation time and make sure that no residual particulates</li> </ul> <p>2.1 Ethanol is not added into the lysate before transferring the sample mixture into FSTG Mini Column</p> <ul style="list-style-type: none"> <li>• Repeat the extraction procedure with a new sample</li> </ul> <p>3.1 Incorrect preparation of Wash Buffer -Ethanol is not added into Wash Buffer when first open</p> <ul style="list-style-type: none"> <li>• Make sure that the correct volumes of ethanol (96-100%) is added into Wash Buffer when first open.</li> <li>• Repeat the extraction procedure with a new sample.</li> </ul> <p>3.2 The volume or the percentage of ethanol is not correct before adding into Wash Buffer</p> <ul style="list-style-type: none"> <li>• Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open</li> <li>• Repeat the extraction procedure with a new sample</li> </ul> |
| <p>Column is clogged</p>  | <p>1.1 Lysate contains insoluble residues</p> <ul style="list-style-type: none"> <li>• Remove insoluble residues (e.g. filter paper, bone or hari) before centrifuging.</li> </ul> <p>1.2 Sample is too viscous</p> <ul style="list-style-type: none"> <li>• Reduce the sample volume</li> </ul> <p>1.3 Insufficient activity of proteinase K</p> <ul style="list-style-type: none"> <li>• Use a fresh or well-stored Proteinase K stock solution.</li> <li>• Repeat the extraction procedure with a new sample. Do not add Proteinase K into FSTG2 buffer directly.</li> </ul>   |



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| <p>Poor quality of genomic (DNA <math>A_{260}/A_{280}</math> ratio of eluted DNA is low)</p> | <p>1.1 Poor Cell Lysis-because of insufficient Proteinase K activity</p> <ul style="list-style-type: none"> <li>Repeat the extraction procedure with a new sample. Use a fresh or well store Proteinase K stock solution.</li> <li>Don't add Proteinase K Directly to FSTG2 buffer.</li> </ul> <p>1.2 Poor Cell Lysis-because of insufficient mixing with FSTG2 buffer</p> <ul style="list-style-type: none"> <li>Repeat the extraction procedure with a new sample. Mix the sample and FSTG2 Buffer immediately and thoroughly by pulse-vortexing.</li> </ul> <p>1.3 Poor Cell Lysis-because of insufficient incubation time</p> <ul style="list-style-type: none"> <li>Repeat the extraction procedure with a new sample. Extend the Incubation time and make sure that no residual particulates remain.</li> </ul> <p>2.1 Ethanol is not added into the lysate before transferring the sample mixture into FSTG Mini Column</p> <ul style="list-style-type: none"> <li>Repeat the extraction procedure with a new sample</li> </ul> <p>3.1 Incorrect preparation of Wash Buffer -Ethanol is not added into Wash Buffer when first open</p> <ul style="list-style-type: none"> <li>Make sure that the correct volumes of ethanol (96-100%) is added into Wash Buffer when first open.</li> <li>Repeat the extraction procedure with a new sample.</li> </ul> <p>3.2 The volume or the percentage of ethanol is not correct before adding into Wash Buffer</p> <ul style="list-style-type: none"> <li>Make sure that the correct volumes of ethanol (96-100%) is added into wash buffer when first open</li> <li>Repeat the extraction procedure with a new sample</li> </ul> <p>3.3 Genomic DNA is contaminatd</p> <ul style="list-style-type: none"> <li>Don't wet the rim of the column during sample and buffer lodading.</li> </ul> |
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| <p>Poor quality of genomic (DNA-A /A ratio of eluted DNA is <sup>high</sup>)</p> | <p>1.1 A lot of residual RNA in eluted DNA</p> <ul style="list-style-type: none"> <li>Follow the general protocol step 6 to remove RNA</li> </ul> <p>1.2 FSTG2 Buffer added to the sample before adding RNase A</p> <ul style="list-style-type: none"> <li>Make sure that RNase A has been added to the sample before adding FSTG2 Buffer when using optional RNase A step.</li> </ul> |
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| <p>Degradation of eluted DNA</p> | <p>1.1 Sample is old</p> <ul style="list-style-type: none"> <li>Always use fresh or well-stored sample for genomic DNA extraction</li> </ul> <p>1.2 Buffer for gel electrophoreses contaminated with Dnase</p> <ul style="list-style-type: none"> <li>Use fresh running buffer for gel electrophoresis</li> </ul> <p>1.3 Buffer for gel electrophoresis contaminated with Dnase</p> <ul style="list-style-type: none"> <li>Genomic DNA extracted from paraffin-embedded tissue is usually It is still suitable for PCR reaction, but is not recommended for Southern blotting and restriction analysis</li> </ul> |
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