BIO-DOC

Tissue Genomic DNA Extraction Mini Kit (With Proteinase K)

Only for Research.

For extraction genomic DNA from animal cells, animal tissues, blood, bacteria, paraffin fixed tissue, yeast and fungi

Kit Contents:

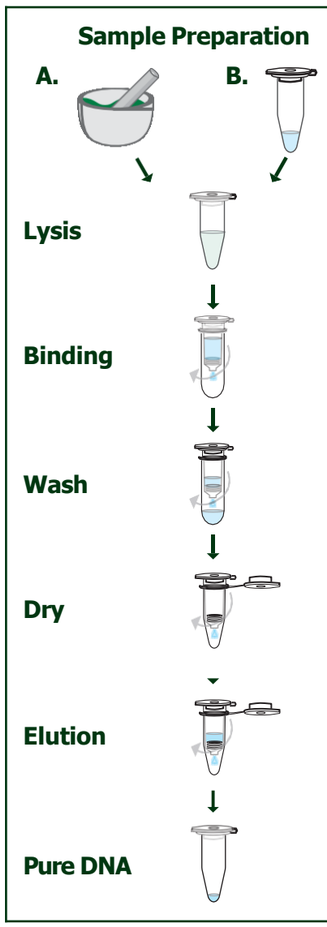
Cat. No:	BDCK006-004 (4 preps_sample)	BDCK006-050 (50 preps)	BDCK006-100 (100 preps)	BDCK006-200 (200 preps)
TG1 Buffer	1.5 mL	15 mL	30 mL	60 mL
TG2 Buffer	1.5 mL	15 mL	30 mL	60 mL
Proteinase K (lyophilized)[a]	1 mg	11 mg	11 mg x 2	11 mg x 4
GW Buffer (concentrate)[b]	1.3 mL	22 mL	44 mL	88 mL
Wash Buffer (concentrate)[c]	1 mL	10 mL	20 mL	40 mL
Elution Buffer	1 mL	15 mL	30 mL	60 mL
TG Mini Column	4 pcs	50 pcs	100 pcs	200 pcs
Collection Tube	8 pcs	100 pcs	200 pcs	400 pcs
Elution Tube	4 pcs	50 pcs	100 pcs	200 pcs
User Manual	1	1	1	1
※**Preparation of Proteinase K solution (10 mg/mL) by adding ddH_2O**				
ddH_2O volume for Proteinase K[a]	0.1 mL	1.1 mL		
※**Preparation of GW Buffer and Wash Buffer by adding ethanol (96～100%)**				
Ethanol volume for GW Buffer[b]	0.5 mL	8 mL	16 mL	32 mL
Ethanol volume for Wash Buffer[c]	4 mL	40 mL	80 mL	160 mL

Specification:

Principle:	mini spin column (silica matrix)
Sample size:	1. Up to 25 mg animal tissue 2. 1.2 cm mouse tail 3. Up to 10^7 cultured cells
Operation time:	30～60 minutes
Binding capacity:	up to 60 µg total DNA / column
Expected yield:	15 ~35 µg / prep
Column applicability:	centrifugation and vaccum
Minimum elution volume:	50 µl

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_2O to Proteinase K** tube to make a **10 mg/mL stock solution**.
   Make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 ℃** .
3. **Add ethanol (96- 100 %) to GW Buffer and Wash Buffer when first open.**
4. Prepare dry baths or water baths before the operation. One to 60℃ for step 2.1 and the other to 70℃ for step 2.2.
5. Preheat the Elution Buffer to 70℃ for step 7.
6. All centrifuge steps are done at full speed(～ 18,000 x g) in a microcentrifuge.



A. For Tissue Sample : Homogenization
(Grind the sample in liquid nitrogen).

B. For Cultured cells : Centrifuge at 300 x g for 5 min at 4°C.

1. **Add 200 µl TG1 Buffer.**
2. **Add 20 µl Proteinase K (10mg/mL).**
3. **Add 200 µl TG2 Buffer.**
4. **Add 200 µl ethanol (96-100%).**

Centrifuge at full speed (~18,000 x g) for 1 min.

1. **Add 500 µl of GW Buffer.** Centrifuge at full speed (~18,000 x g) for 1 min.
2. **Add 750 µl of Wash Buffer.** Centrifuge at full speed (~18,000 x g) for 1 min. Repeat this step for one more washing.

Centrifuge at full speed (~18,000 x g) for an additional 3 min to dry the column.

Add 100 µl of preheated Elution Buffer or ddH_2O (pH 7.5-9.0)
Centrifuge at full speed (~18,000 x g) for 2 min.

General Protocol: For Animal Tissues

Please Read Important Notes Before Starting Following Steps.

STEP	PROCEDURE
1 Sample preparation	Cut up to 25 mg tissue sample to a microcentrifuge tube (not provided). Grind the tissue sample in liquid nitrogen with mortar and pestle then transfer the powder to a microcentrifuge tube.
2.1 Lysis	**1. Add 200 µl TG1 Buffer** and mix well by Micropestle or pipette tip. **2. Add 20 µl Proteinase K (10mg/mL)** to the sample mixture. Mix thoroughly by vortexing. 3. Incubate at 60℃ until the tissue is lysed completely (1~3 h). Vrotex occasionally during incubation. **Note:** Sample can be incubated overnight as well for complete lysis. **Optional:** If RNA-free genomic DNA is required, **add 4 µl of 100 mg /mL RNase A** (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 min.
2.2 Lysis	**Add 200 µl TG2 Buffer** to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70℃ for 10 min.
3 Ethanol Dilution	**Add 200 µl ethanol (96-100%)** to the sample mixture. Mix immediately and thoroughly by vortexing to yield a homogeneous solution.
4 DNA Binding	Place a TG Mini Column in a Collection Tube. Transfer the mixture (including any precipitate) carefully to the TG Mini Column. Centrifuge at full speed (~18,000 x g) for 1 min then place the TG Mini Column to a new Collection Tube.
5.1 Wash	**Add 400 µl GW Buffer** to the TG Mini Column. Centrifuge at full speed for 1 min then discard flow-through.
5.2 Wash	**Add 750 µl Wash Buffer** to the TG Mini Column. Centrifuge at full speed for 1 min then discard flow-through.

6 Dry column	Centrifuge the TG Mini Column at full speed for an additional 3 min to dry the TG Mini Column.
7 DNA Elution	**1. Add** 100 µl of **preheated Elution Buffer or ddH₂O (pH 7.5-9.0)** to the membrane of the TG Mini Column. Stand the TG Mini Column for 3 min. 2. Centrifuge at full speed for 2 min to elute DNA.

Special Protocol: The sample preparation For Animal Cultured Cells

Additional requirement	1. RNase A (optional) 2. 96~100% ethanol 3. trypsine or cell scraper (for monolayer cell) 4. PBS
Method	**Harvest cells** **1. For Cells grown in suspension** a. Transfer the appropriate number of cell (up to 1 x 10^7) to a microcentrifuge tube. b. Centrifuge at 300 x g for 5 min. Discard supernatant carefully and completely. **For Cells grown in monolayer** a. Detach cells from the dish or flask by trypsinization or using a cell scraper. Transfer the appropriate number of cell (up to 1 x 10^7) to a microcentrifuge tube. b. Centrifuge at 300 x g for 5 min. Discard supernatant carefully and completely. 2. Resuspend cell pellet in PBS to a final volume of 200 µl. 3. Follow the Animal Tissuel Protocol starting from step 2.1 Lysis.

Special Protocol: The sample preparation For Blood

Additional requirement	1. RNase A (optional) 2. 96~100% ethanol 3. PBS
Method	1. Transfer up to 200 µl sample (whole blood, serum, plasma, body fluids, buffy coat) to a microcentrifuge tube. • **If the sample volume is less than 200 µl , add the appropriate volume of PBS.** **Optional:** If RNA-free genomic DNA is required, **add 4 µl of 100 mg/mL RNase A (not provided).** Mix thoroughly by vortexing and incubate at room temperature for 2 min. **2. Add 20 µl Proteinase K** to the sample, and then **add 200 µl TG2 Buffer** to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60℃ for 30 min. Vrotex occasionally during incubation. 3. Incubate at 70℃ for 10 min. 4. Follow the Animal Tissuel Protocol starting from step 3. Ethanol Dilution.

Special Protocol: The sample preparation For Bacteria

Additional requirement	1. RNase A (optional) 2. 96~100% ethanol 3. For Gram-positive bacteria: lysozyme reaction solution (20 mg/mL lysozyme; 20 mM Tris-HCI, pH 8.0; 2mM EDTA; 1.2 %Triton)
Method	**1. For bacterial cultures** a. Transfer 1 mL well-grown bacterial culture to a microcentrifuge tube (not provided). b. Descend the cells by centrifuging at full speed for 2 min and discard supernatant completely. c. Follow the Animal Tissue Protocol starting from step 2.1 Lysis. **For bacterial in biological fluids** a. Collect cells by centrifuging biological fluids at 7,500 rpm (5,000 x g) for 10 min and discard supernatant completely. b. Follow the Animal Tissue Protocol starting from step 2.1 Lysis. **For bacteria from eye, nasal, pharyngeal, or other swabs** a. Soak the swabs in 2 mL PBS at room temperature for 2- 3 hr. b. Collect cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min and discard supernatant completely. c. Follow the Animal Tissue Protocol starting from step 2.1 Lysis. **For Gram-positive bacteria** **HINT: Set dry or water baths: one to 37℃ , another to 60℃ and the other to 95℃ .** a. Transfer 1 mL well-grown bacterial culture to a microcentrifuge tube (not provided). b. Descend the cells by centrifuging at full speed for 2 min and discard supernatant completely. c. Resuspend the cell pellet in **200 µl lysozyme reaction solution** (20 mg/mL lysozyme; 20 mM Tris-HCI, pH 8.0; 2mM EDTA; 1.2 %Triton). Incubate at 37℃ for 30~60 min. **Optional:** If RNA-free genomic DNA is required, add 4 µl of 100 mg/mL RNase A (not provided). Mix thoroughly by vortexing and incubate at room temperature for 2 min. **2. Add 20 µl Proteinase K** to the sample, and then **add 200 µl TG2 Buffer** to the sample. Mix thoroughly by pulse-vortexing. Incubate at 60℃ for 30 min and vrotex occasionally during incubation. **Optional:** If required, incubate at 95℃ for 15 min to inactivate pathogens. **Note:** that incubation at 95℃ can lead to some DNA degradation. 3. Follow the Animal Tissue Protocol starting from step 3. Ethanol Dilution.

Special Protocol: The sample preparation For Yeast

Additional requirement	1. xylene & ethanol (96~100%) 2. liquid nitrogen 3. Mortar a rotor-stator homogenizer or a 20-G needle syringe 4. ß-Mercaptoethanol 5. 70% RNase-free ethanol
Method	1. Transfer 3 mL log-phase (OD600 = 10) yeast culture to a microcentrifuge tube (not provided). 2. Descend the cells by centrifuging at 7,500 rpm (5,000 x g) for 10 min. Discard supernatant completely. 3. Resuspend the cell pellet in **600 µl sorbitol buffer** (1M sorbitol; 100 mM EDTA; 14 mM ß-mercaptoethanol). **Add 200 U zymolase or lyticase** and incubate at 30℃ for 30 min. 4. Centrifuge at 7,500 rpm (5,000 x g) for 5 min. Remove supernatant by pipetting. 5. Follow the Animal Tissue Protocol starting from step 2.1 Lysis.

Special Protocol: The sample preparation For Dried Blood Spot

Additional equipment	1. RNase A (optional) 2. 96~100% ethanol
Method	1. Cut the filter paper (e.g. S&S903) with dried blood spot into a microcentrifuge tube. Add **200 µl TG1 Buffer** and incubate at 85℃ for 10 min. **2. Add 20 µl Proteinase K** to the sample mixture. Mix thoroughly by vortexing. Incubate at 60℃ for 1 hr. Vrotex occasionally during incubation. 3. Follow the Animal Tissue Protocol starting from step 2.2 Lysis.

Special Protocol: The sample preparation For Fixed Tissue

Additional equipment	1. RNase A (optional) 2. 96~100% ethanol 3. Xylene - for paraffin-embedded tissues
Method	**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 supernatant by pipetting. **4. Add 1 mL ethanol (96- 100 %)** to the deparaffined tissue, mix gently by vortexing. 5. Centrifuge at full speed for 3 min. Remove supernatant by pipetting. 6. Repeat step 4 and 5. 7. Incubate at 37℃ for 10 ~15 min to evaporate ethanol residue completely. 8. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from step 2.1 Lysis. **For formalin-fixed tissues** 1. Wash 25 mg tissue sample twice with 1 mL PBS to remove formalin. 2. Grind the tissue sample by micropestle or liquid nitrogen and follow the Animal Tissue Protocol starting from step 2.1 Lysis.

TEL : 886-2-2228-8950
FAX : 886-2-2228-8955
Mail : info@lis-tek.com
4F., No.926, Zhongzheng Rd., Zhonghe Dist., New Taipei City 235, Taiwan (R.O.C.)
www.lis-bio.com/zh-TW