CatchGene

CatchGene™ Tissue DNA Kit

Cat. No.	Rxn
MD20004	4
MD20050	50
MD20250	250

Kit Description

CatchGene™ Tissue DNA Kit is a very broad spectrum kit. It's able to purify gDNA from a variety of samples such like different animal tissues, dried blood spot, nails, hair, feathers, bacterial cultures, bacterial in biological fluids, gram-positive bacteria, avian blood, Insect, urine, etc. Our Buffer DTL is a very strong lysis buffer, combine with proper homogenization, it's able to lysis big tissue block into smaller tissue pieces or even cells. Combine with Buffer DL, to lysis cells thoroughly and adjust to best binding condition. CatchGene™ Tissue DNA Kit is able to get high yield of gDNA in high purity.

Kit Content

	4 rxn	50 rxn	250 rxn	
Spin Column	4	50	250	pcs
Collection Tube (2 ml)	12	150	750	pcs
PK Solvent	1.5	1.5	7.5	ml
Proteinase K	1	11	55	mg
Buffer DTL*	1.92	24	120	ml
Buffer DL*	0.96	12	60	ml
Buffer W1 (concentrated)	1.68	21	105	ml
Buffer W2 (concentrated)	0.68	8.4	42	ml
Elution Buffer	0.96	12	60	ml

Kit Storage

Upon arrival,

1. Please store **Proteinase K** at **-20** ℃ for long term storage.

Buffer, solvent and consumables, please store at 15-25 ℃.

*There might precipitate in Buffer DTL or Buffer DL if stored at low temperature. Dissolve it by incubation the buffer at 56°C for 10 mins.

Kit Preparation

1. **Prepare 10 mg/ml Proteinase K**
   For 1 mg Proteinase K, please add 100 µl PK solvent into tube and vortex thoroughly for dissolving.
   For 11 mg Proteinase K, please add 1100 µl PK solvent into tube and vortex thoroughly for dissolving.
   For 55 mg Proteinase K, please add 5.5 ml PK solvent into tube and vortex thoroughly for dissolving.
   After dissolving into solvent, plase store in 4°C for 6 month or -20°C for 1 year.
2. **Prepare Buffer W1**
   Add equal volume of 100% EtOH into Buffer W1 (concentrated) to get Buffer W1.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.
3. **Prepare Buffer W2**
   Add 4 volume of 100% EtOH into Buffer W2 (concentrated) to get Buffer W2.
   After adding 100% EtOH, please check the sticker on the bottle and close the cap tightly.

General Protocol

1. Weight up to 25 mg of animal tissue or no more than 10 mg spleen tissue.
2. Homogenize tissue samples by one of following methods.
   A. Cut tissue samples into small pieces.
   Place into a 2 ml micro-centrifuge tube (not provided). Proceed with step 3.
   B. Homogenize tissue sample with liquid nitrogen.
   Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle. Proceed with step 3.
   C. Homogenize tissue sample with buffer.
   Place tissue sample into 2 ml micro-centrifuge tube (not provided) containing 100 µl PBS. Homogenize samples with homogenizer thoroughly. Add 150 µl Buffer DTL and proceed with step 4.
3. Add 250 µl Buffer DTL, vortex vigorously for 30 sec.
4. Add 20 µl Proteinase K, vortex for 15 sec then incubate at 60 °C for 15 min or until all tissue lysed properly. (vortex periodically in order to increase lysis efficiency)
5. Centrifuge at 11,000 x g for 3 min, transfer 200 µl supernatant (Avoid aspirate any debris.) to a new 1.5 ml micro-centrifuge tube. (If the volume of supernatant is under 200 µl, please compensate with Buffer DTL to 200 µl.)
6. Add 200 µl Buffer DL, vortex for 15 sec. Incubate at 70 °C for 10 min. Cool down to room temperature and brief spin down.
7. Add 200 µl 100% EtOH, vortex for 15 sec then brief spin down.
8. Transfer all lysate to the Spin Column (with 2ml Tube), centrifuge at 11,000 x g for 1 min.
9. Carefully move the Spin Column to a new Collection Tube (2 ml).
10. Add 700 µl Buffer W1 into the Spin Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
11. Add 700 µl Buffer W2 into the Spin Column, centrifuge at 11,000 x g for 1 min, discard all wash buffer.
12. Carefully move the Spin Column to a new Collection Tube (2 ml), centrifuge at full speed (~18,000 x g) for 3 min.
13. Place the spin column into 1.5 ml micro-centrifuge tube, add 30-200 µl Elution Buffer and incubation at room temperature for 3 min, centrifuge at 11,000 x g for 1 min for elution.

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Sample Pretreatment

- **Avian blood**
    1. Transfer 10 µl avian blood into a 1.5 ml micro-centrifuge tube.
    2. Add 90 µl PBS into tube.
    3. Add 100 µl Buffer DTL into tube and vortex for 10 sec, brief spin down.
    4. Add 20 µl Proteinase K, vortex for 10 sec, brief spin down then incubate at 60 °C for 15 min.
    5. Proceed to **step 6 of General Protocol**.
- **Bacteria cultures**
    1. Transfer 1 ml well-grown bacterial culture (up to $1x10^9$ cells) to a micro-centrifuge tube (not provided).
    2. Descend the bacterial cells by centrifuging at 11,000 x g for 2 min and discard the supernatant thoroughly.
    3. Proceed to **step 3 of General Protocol**.
- **Bacteria in body fluids**
    1. Collect bacteria by centrifuging biological fluids at 11,000 x g for 10 min.
    2. Discard the supernatant thoroughly.
    3. Proceed to **step 3 of General Protocol**.
- **Buccal Swab**
    1. Place the Swab into a 2 ml sample tube (not provided) and cut the stick of swab at proper length in order to close the lid of tube.
    2. Add 400 µl Buffer DTL and 400 µl Buffer DL to the sample then mix by vigorously vortex for 30 sec. Brief spin down. *Extra Buffer DL need to be purchased in this application, please contact with your distributor for ordering.
    3. Add 20 µl Proteinase K, vortex for 30 sec then incubate at 60 °C for 30 min.
    4. Centrifuge at 11,000 x g for 2 min then transfer 400 µl lysate to a new tube (Avoid to aspirate any debris).
    5. Proceed to **step 7 of General Protocol**.
- **Buffy Coat**
    1. Add 25 µl Proteinase K (10 mg/ml) into a 1.5 ml micro-centrifuge tube.
    2. Transfer 25 µl of buffy coat sample and add 225 µl ddH_2O into the tube, vortex for 15 sec.
    3. Add 250 µl Buffer DTL, vortex vigorously for 30 sec then incubate at 60 °C for 15 min.
    4. Proceed to **step 5 of General Protocol**.
- **Dried blood spot**
    1. Place 1 piece of 5mm diameter or 3 pieces of 3mm diameter dried blood spot into a 1.5 ml micro-centrifuge tube.
    2. Add 220 µl Buffer DTL into tube and vortex for 10 sec, brief spin down.
    3. Incubate at 85°C for 10 min, brief spin down.
    4. Add 20 µl Proteinase K, vortex for 10 sec, brief spin down then incubate at 60 °C for 60 min.
    5. Add 200 µl Buffer DL, vortex for 15 sec. Incubate at 70 °C for 10 min, brief spin down.
    6. Transfer 400 µl supernatant to a new tube. Avoid aspirating any blood spot or debris. (If the lysate volume is less than 400 µl ,compensate to 400 µl by Buffer DTL.)
    7. Add 1 µg Carrier RNA (not provided) into the lysate. (Carrier RNA is not included in this kit. Please contact your supplier to buy it to get best recovery of gDNA from dried blood spot sample.)
    8. Proceed to **step 7 of General Protocol**.
- **Feathers**
    1. Cut a 2-3 cm piece from the feather** (up to 25 mg), and transfer it to a 1.5ml micro-centrifuge tube (not provided).
       **For small birds, such as finches, use primary feathers (e.g., the largest tail or wing feathers). For medium birds, such as canaries, secondary tail or wing feathers can be used. For large birds, such as chickens or cockatoos, breast or back feather can be used.
    2. Add 20 µl 1M DTT (not provided) into the tube.
    3. Proceed to **step 3 of General Protocol**.
- **Nails**
    1. Cut the sample (up to 25 mg) into small pieces and place into a 1.5ml micro-centrifuge tube (not provided).
    2. Add 20 µl 1M DTT (not provided) into the tube.
    3. Proceed to **step 3 of General Protocol**.
- **Urine**
    1. Centrifuge 10-50 ml urine sample at 2,500 x g for 10 minute.
    2. Discard the supernatant carefully then add 200 µl Buffer DTL and 200 µl Buffer DL into the tube.
    3. Suspend the pellet by buffers then transfer all sample into a new 2 ml micro-centrifuge tube.
    4. Add 20 µl Proteinase K, vortex for 30 sec, brief spin down then incubate at 60 °C for 20 min.
    5. Centrifuge at 11,000 x g for 1 min and transfer 400 µl supernatant to a new tube. Avoid aspirating any stones or cell debris.
    6. Proceed to **step 7 of General Protocol**.

Sample Pretreatment

- **Yeast**
  1. Transfer 1 ml log-phase (O.D.600=1) yeast culture to a 2ml Sample Tube. Do not load more than 2 x 10^7 cells. Overloading might cause bad in yield and purity.
  2. Descend the yeast cells by centrifuging at 5,000 x g for 5 min and discard the supernatant completely. Carefully remove any remaining media by aspiration. Remaining media will affect digestion of the cell wall.
  3. Re-suspend the cell pellet in 200 µl sorbitol buffer (1M sorbitol; 100 mM EDTA; 0.1% ß-mercaptoethanol). (Please add ß-me into the sorbitol buffer freshly.)
  4. Add 20-200 U zymolase or lyticase (not provided), incubate at 30 °C for 30 min.
  5. Descend spheroplasts by centrifuging at 5,000 x g for 5 min and carefully remove the supernatant thoroughly.
  6. Proceed to **step 3 of General Protocol**. (For different strain, the 60 °C incubation time might different.)

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