iCatcher® Tissue DNA 250 Kit

Cat. No.	Rxn
AD20025-36	36

Kit Content

	36rxn	
Syringe	36	set
Elution Tube	36	pcs
AD20025 Cartridge	36	set
AD20025 Column Set	36	set
AD20025 Tip Set	36	set
EtOH Tube	36	pcs
Sample Tube	36	pcs
Buffer DTL	10	ml
Proteinase K	11	mg
PK Solvent	1.5	ml

Kit Storage

Upon arrival,

1. Please store **Proteinase K** at **-20 ℃** for long term storage.
2. Solvent and consumables, please store at 15-25 ℃.

Kit Preparation

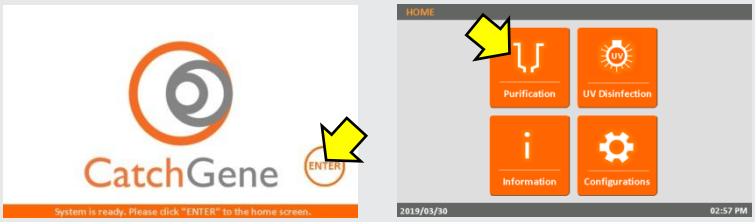
1. **Prepare 10 mg/ml Proteinase K**
   For 11 mg Proteinase K, please add 1.1 ml PK Solvent into tube and vortex thoroughly for dissolving.
   After dissolving into solvent, plase store in 4°C for 6 month or -20°C for 1 year.

General Pretreatment for Tissue Sample

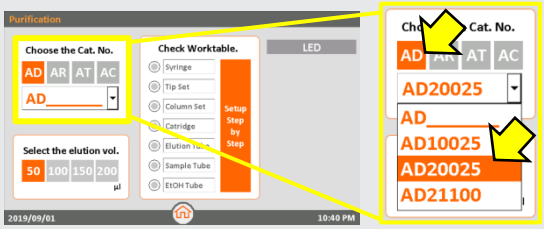
1. Weight up to 25 mg of animal tissue or no more than 10 mg spleen tissue.
2. Homogenize tissue samples by one of following methods.
   A. Homogenize tissue sample with liquid nitrogen.
   Grind tissue sample thoroughly with liquid nitrogen by beads beater, tissue homogenizer or mortar & pestle. Proceed with step 3.
   B. 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. (If the lysate volume is less than 250 µl ,compensate to 250 µl by Buffer DTL.)
4. Add 25 µl Proteinase K, vortex for 15 sec then incubate at 60 °C for 15 min or until all tissue lysed properly.
5. Centrifuge at 11,000 x g for 3 min.
6. Transfer 250µl of supernatant into the Sample Tube.

Step by Step to start a AD20025 Purification Run

1. On the **Start** screen: Click “ENTER” button to enter the HOME screen.
2. On the **HOME** screen: Click “Purification” icon to start a purification run.

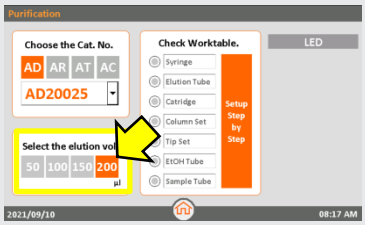


3. Please choose **Cat. No.**



Please click “**AD**”
Then choose “**AD20025**”
For **iCatcher® Tissue DNA 250 Kit**

4. Choose **Elution Vol.**



We suggest to elute in **50-200µl** for iCatcher® Tissue DNA 250 Kit.

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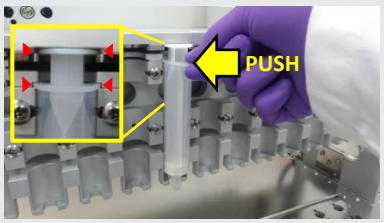
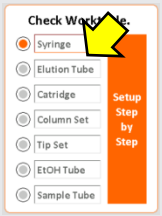
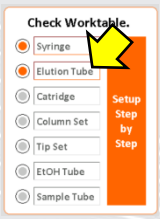
5. Insert the **Syringe** into the groove of Syringe Seat and push it to the end.



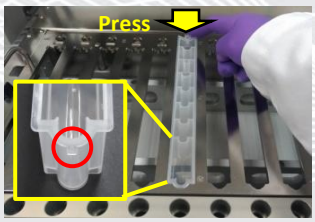
Check the **Syringe**.

6. Labeling , then open the lid and place the **Elution Tube** on the Elution Tube position.

Check the **Elution Tube**.

7. Insert the front protrude part of **Cartridge** into Cartridge position and press the bottom down. Then remove the foil.



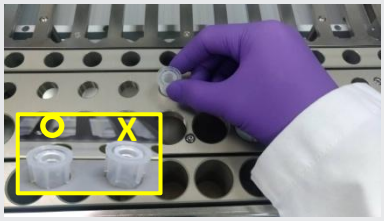
Check the **Cartridge**.

Important! Please must **remove the foil** before running a protocol.

8. Insert **Column Set** into Column Set position and press into bottom.

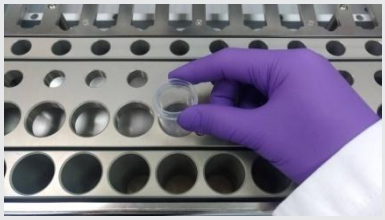

Check the **Column Set**.

9. Place **Tip Set** on Tip Set position and press into bottom.

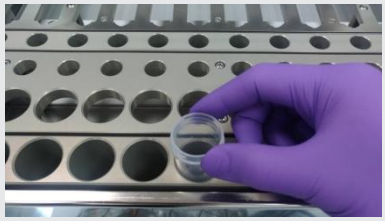
Check the **Tip Set**.

10. Add **10 ml** 100% EtOH into **EtOH Tube** and place on the EtOH Tube position.


Add **10ml** 100% EtOH for AD20025
Check the **EtOH Tube**.

11. Prepare sample and load the Sample Tube into the Sample Tube position.


Check the **sample Tube**.
Click "**Go**" to start purification.

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iCatcher® Tissue DNA 250 Kit - Appendix I

Pretreatment of Other Samples

- **Amniotic Fluid**
  1. Transfer 10 ml amniotic fluid into a 15 ml centrifuge tube.
  2. Descend cells by centrifuging at 300 x g for 10 min.
  3. Leave 150 µl of the supernatant (Avoid to disturb the pellet).
  4. Add 150 µl Buffer DTL into tube and vortex for 10 sec, brief spin down.
  5. Transfer mixture to a new 1.5 ml micro-centrifuge tube.
  6. Add 25 µl Proteinase K, vortex for 10 sec, brief spin down then incubate at 60 °C for 30 min.
  7. Centrifuge at 11,000 x g for 2 min then transfer 250 µl lysate to the Sample Tube (Avoid to aspirate any debris).
  8. Load the Sample Tube into the Sample Tube position of iCatcher.

- **Buffy Coat**
  1. Add 25 µl Proteinase K (10 mg/ml) into the bottom of Sample Tube.
  2. Transfer 25 µl of buffy coat sample into 2 ml micro-centrifuge tube.
  3. Add 250 µl Buffer DTL, vortex vigorously for 30 sec then incubate at 60 °C for 15 min.
  4. Centrifuge at 11,000 x g for 3 min.
  5. Transfer 250 µl of supernatant into the Sample Tube.

- **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 250 µl PBS and 250 µl Buffer DTL to the sample then mix by vigorously vortex for 30 sec. Brief spin down.
  3. Add 25 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 250 µl lysate to the Sample Tube (Avoid to aspirate any debris).
  5. Load the Sample Tube into the Sample Tube position of iCatcher.

- **Culture cell**
  1. To collected the cells depend on cells growing type (cell number recommended not more than 1 x 10^7)
  2. Harvested the cells grown in suspension by centrifuging for 5 min at 300 x g. Carefully remove supernatant.
  3. Add 250 µl Buffer DTL, vortex vigorously for 30 sec.
  4. Add 25 µl Proteinase K, vortex for 15 sec, then incubate at 60 °C for 15 min.
  5. Centrifuge at 11,000 x g for 3 min.
  6. Transfer 250µl of supernatant into the Sample Tube.

- **Swab in 0.5 ml CatchGene Preservation Buffer Tube**
  1. Add 25 µl Proteinase K, vortex for 30 sec then incubate at 60 °C for 1 hr.
  2. Centrifuge at 11,000 x g for 2 min then transfer 250 µl lysate to the Sample Tube (Avoid to aspirate any debris).
  3. Load the Sample Tube into the Sample Tube position of iCatcher.

- **Dried blood spot**
  1. Place 3 piece of 5 mm diameter or 3 pieces of 3 mm diameter dried blood spot into a 2 ml micro-centrifuge tube.
  2. Add 250 µl Buffer DTL into tube and vortex for 30 sec, brief spin down.
  3. Incubate at 85°C for 10 min, brief spin down and cool down to room temperature.
  4. Add 25 µl Proteinase K, vortex for 30 sec, brief spin down then incubate at 60 °C for 60 min.
  5. Transfer lysate and dried blood spot to the Filter Column with 2 ml micro-centrifuge tube. Centrifuge at 11,000 x g for 3 min.
  6. Aspire 250 µl lysate to a iCatcher Sample Tube. (If the lysate volume is less than 250 µl ,compensate to 250 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. Load the Sample Tube into the Sample Tube position of iCatcher.

- **Saliva**
  1. Centrifuge saliva sample at 100 x g for 1 min, then aliquot 250 µl to the Sample Tube (Avoid to aspirate any debris).
  2. Add 25 µl Proteinase K (10 mg/ml) into the Sample Tube.
  3. Load the Sample Tube into the Sample Tube position of iCatcher.

- **Whole Blood**
  1. Add 25 µl Proteinase K (10 mg/ml) into the bottom of Sample Tube.
  2. Transfer 250 µl of whole blood sample into the Sample Tube.
  3. Load the Sample Tube into the Sample Tube position of iCatcher (no need to mix or pipette it).

- **Cultured Bacteria**

Bacteria in agar

1. Add 250 µl Buffer ST1/DTL into the Beads Tube. (Buffer ST1 and the Beads Tube are not included in this kit, if needed please contact your supplier for purchasing.)
2. Pick up one drop of cultured bacteria and mix with Buffer ST1, vortex vigorously for 1 min, then brief spin down.
3. Incubate at 95°C for 15 min, then cool down to room temperature. Brief spin down.
4. Add 25 µl Proteinase K, vortex for 15 sec, brief spin down then incubate at 60 °C for 30 min. (vortex periodically in order to increase lysis efficiency)
5. Centrifuge at 11,000 x g for 3 min, transfer 250 µl supernatant (Avoid aspirate any debris.) to the Sample Tube.
6. Load the Sample Tube into the Sample Tube position of iCatcher.

Bacteria in medium

1. Transfer up to 0.5 ml well-grown bacterial culture (up to $1x10^8$ 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. Add 300 µl Buffer ST1/DTL to suspend bacterial pellet, then transfer all lysate into the Beads Tube. Vortex vigorously for 1 min, then brief spin down. (Buffer ST1 and the Beads Tube are not included in this kit, if needed please contact your supplier for purchasing.)
4. Incubate at 95°C for 15 min, then cool down to room temperature. Brief spin down.
5. Add 25 µl Proteinase K, vortex for 15 sec, brief spin down then incubate at 60 °C for 30 min. (vortex periodically in order to increase lysis efficiency)
6. Centrifuge at 11,000 x g for 3 min, transfer 250 µl supernatant (Avoid aspirate any debris.) to the Sample Tube.
7. Load the Sample Tube into the Sample Tube position of iCatcher.