

Kit Content

	36rxn	
Syringe	36	set
Elution Tube	36	pcs
AD23025 Cartridge	36	set
AD23025 Column Set	36	set
AD23025 Tip Set	36	set
EtOH Tube	36	pcs
Sample Tube	36	pcs
Beads Tube	36	set
Buffer MB1	20	ml
Proteinase K	11	mg
PK Solvent	1.5	ml

Kit Storage

- Upon arrival,
- Please store **Proteinase K** at **-20 °C** for long term storage.
- Buffer, solvent and consumables, please store at 15-25 °C.
- If a precipitate has formed in Buffer MB1, dissolve by incubating at 60 °C for 10 min.

Kit Preparation

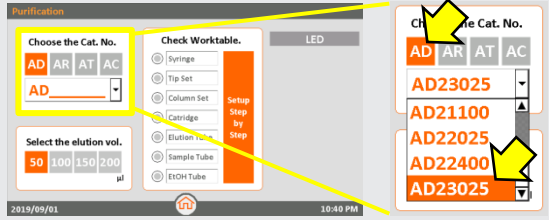
- Prepare 10 mg/ml Proteinase K**
For 11 mg Proteinase K, please add 1100 µl PK Solvent into tube and vortex thoroughly for dissolving. After dissolving into solvent, please store in 4°C for 6 month or -20°C for 1 year.

Step by Step to start a AD23025 Purification Run

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

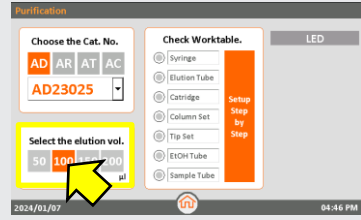


- Please choose **Cat. No.**



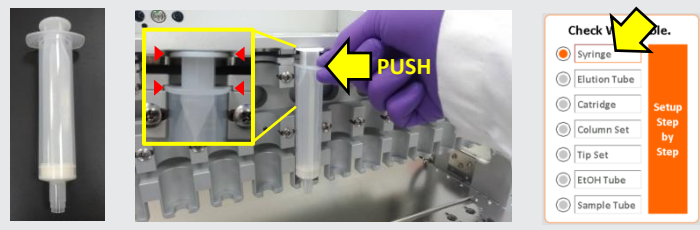
Please click “AD”
Then choose “AD23025”
For iCatcher® Microbe DNA 250 Kit

- Choose **Elution Vol.**



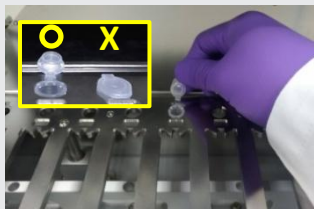
We suggest to elute in **50-200 µl** for Microbe DNA.

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



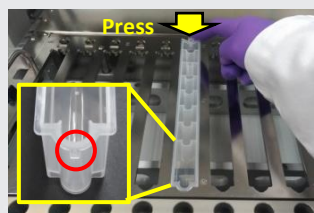
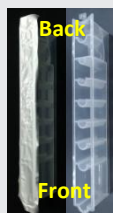
Check the **Syringe**.

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**.

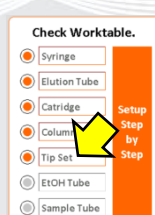
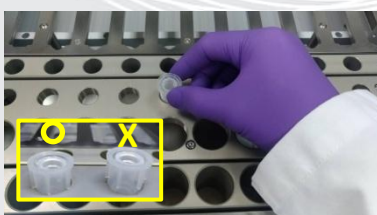
Important! Please must remove the foil before running a protocol.

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



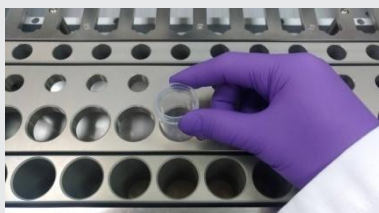
Check the **Column Set**.

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.

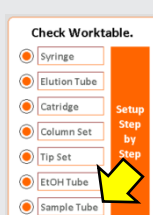
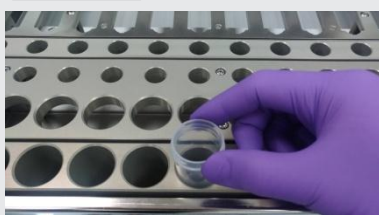


Check the **EtOH Tube**.

Add **10 ml** 100% EtOH into **EtOH Tube**

11. Prepare sample as below,

- Transfer 0.5 -1.5 ml microbial culture or liquid sample to a micro-centrifuge tube (not provided). (For well-grown culture, please transfer up to 0.5 ml culture or no more than 1×10^8 microbial cells)
- Descend the microbial cells by centrifuging at $11,000 \times g$ for 2 min and discard the supernatant thoroughly. (If the pellet is invisible, please leave 10-20 μ l supernatant with the pellet and avoid disturbing the pellet.)
- Add 300 μ l Buffer MB1 to suspend pellet, then transfer all lysate into the "Beads Tube". Vortex vigorously for 1 min, then briefly spin down.
- Incubate at 95°C for 15 min, then cool down to room temperature. Briefly spin down.
- Add 25 μ l 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)
- Centrifuge at $11,000 \times g$ for 3 min, transfer 250 μ l supernatant (avoid aspirate any debris) to the Sample Tube.
- (Optional) Add 4 μ l of 100 mg/ml RNase A (not included) (no need to mix or pipette it)
- Load the **Sample Tube** into the Sample Tube position of iCatcher (no need to mix or pipette it).



Check the **Sample Tube**.

Click "Go" to start purification.

Pretreatment of Other Samples

- **Yeast (Optional process to maximize yield)**
 1. Transfer 1 ml log-phase (O.D.600=1) yeast culture to a 2ml Sample Tube. Do not load more than 2×10^7 cells. Overloading might cause bad in yield and purity.
 2. Descend the yeast cells by centrifuging at $5,000 \times g$ 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 μ l sorbitol buffer (1M sorbitol; 100 mM EDTA; 0.1% β -mercaptoethanol). (Please add β -me into the sorbitol buffer freshly.)
 4. Add 20-200 U zymolyase or lyticase (not provided), incubate at 30 °C for 30 min.
 5. Descend spheroplasts by centrifuging at $5,000 \times g$ for 5 min and carefully remove the supernatant thoroughly.
 6. Proceed to **step 11.c. of General Protocol**. (For different strain, the 60 °C incubation time might different.)

