Bacterial DNA Extraction Kit

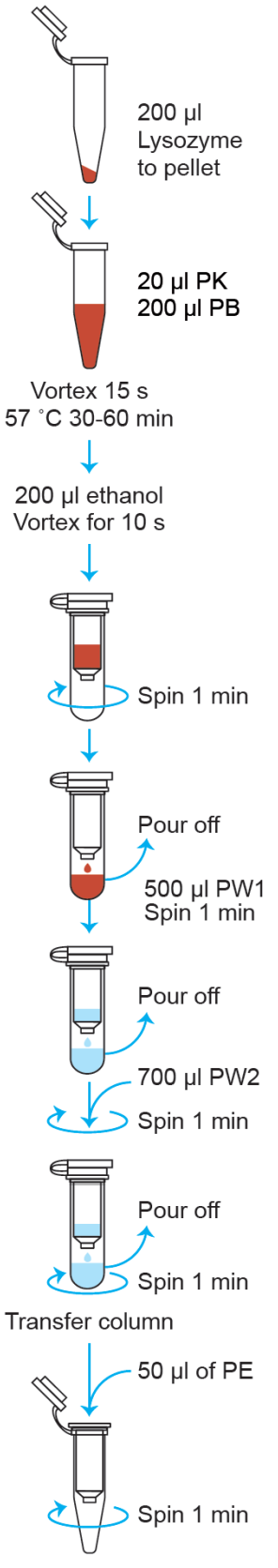
Kit Contents:

Components	50 reactions
PB (Blood Binding Buffer)	12 ml
PW1 (Wash Buffer)	15 ml
PW2 (Wash Buffer	12 ml
PE (Elution Buffer)	12 ml
PK (PK Storage Buffer)	1 ml
Proteinase K	20 mg
Lysozyme Buffer	12 ml
Lysozyme Powder	60 mg
Spin Column	50 pcs
Collection Tube	2 x 50 pcs

Before Starting

1. Add 10 ml of absolute ethanol to the PW1 (only at the first use).
2. Add 48 ml of absolute ethanol to the PW2 (only at the first use).
3. Add Proteinase K (PK) solution to the lyophilized powder of proteinase K and store at -20 ˚C until usage (only at the first use).
4. Add lysozyme powder to the lysozyme buffer and store at -20 ˚C until usage.
   Aliquot to avoid too many freeze-thaw cycles.
5. Preheat the solution of PE to 56 ˚C before starting the extraction process to enhance DNA extraction yield.

Note: PK (PK Storage Buffer), Proteinase K, Lysozyme Buffer and Lysozyme Powder labelled as -20 ˚C for storage conditions, but shipment conditions are +4 ˚C, please put them to -20 ˚C refrigerator when you get the kit.



Protocol:

1. Centrifuge 3-5 ml of bacteria culture media in a 1.5 ml micro tube (13000 RPM, 30 s) and remove supernatants. Add 200 µl Lysozyme solution to the bacterial pellet and Mix them well by vortexing. Incubate at least 30 min at room temperature for Gram positive bacteria or 20 min for Gram negative bacteria.
2. Add 20 µl of proteinase K and 200 µl of PB
3. Mix them well by vortexing (15 s) and incubate at 57 ˚C for 30 min.
4. Add 200 µl of absolute ethanol and mix it by vortexing (10 s).
5. After a quick spin, carefully transfer lysate to the spin column. Do not touch upper rim of column. Spin for 1 min at 13000 rpm.
6. Pour off the flow-through of collection tube.
7. Add 500 µl of PW1 and spin for 1 min at 13000 rpm.
8. Pour off the flow-through of collection tube.
9. Add 700 µl of PW2 and spin for 1 min at 13000 rpm.
10. Pour off the flow-through of collection tube.
11. Repeat step 8 and 9 with 500 µl of PW2 (optional)
12. Spin for 1 min at 13000 rpm to remove the remaining of the wash buffer. Transfer the spin column to a new 1.5 ml micro tube.
13. Add 50 µl of preheated PE, wait 3 min at room temperature or 57 ˚C (If you didn't warm PE). If you want more concentration add less PE (35 µl). Spin for 1 min at 13000 rpm to elute DNA from the column. Store DNA solution at -20 ˚C.