

Qiagen Preperation

1. Hazards

Buffer N3 : Flammable. Skin and eye irritant. If eye contact occurs, rinse with water and seek medical advice.

P2 : Eye irritant. If eye contact occurs, rinse with water and seek medical advice.

PB : Flammable. Skin and eye irritant. If eye contact occurs, rinse with water and seek medical advice.

Waste : N3 and PB contains guanidine hydrochloride which is highly reactive to bleach. Do not add bleach to kit waste.

Protocol Qiagen:

1. Grow 5ml cultures overnight, shaking 225 RPM in LB media with appropriate antibiotic for selection.
2. Centrifuge 3000 rpm for 10 minutes (Jim's centrifuge) to pellet *E. coli*.
3. Resuspend in 250 ul P1 buffer and transfer to microfuge tube.
4. Add 250 ul P2 and invert four times.
5. Add 350 ul N3 and invert four times.
6. Centrifuge 10minutes 13000 rpm (17,000 x g)
7. Decant supernatant into Qiaprep spin column.
8. Centrfuge 60 seconds
9. Add 500 ul PB to spin column
10. Centrfuge 60 seconds and discard eluent
11. Add 750 ul PE to spin column
12. Centrfuge 60 seconds and discard eluent
13. Repeat step 12 and put column in new microfuge tube
14. Add 50ul EB buffer or H2O at pH 8.0
15. Incubate for 60 seconds at room temperature
16. Centrfuge 60 seconds (DNA in eluent)

2. Steps to Avoid injury.

1. Wear gloves
2. Do not work near flame.
3. Work in clutter-free environment

4. What to do with waste or in case of spill.

1. Add bleach to waste LB and pour down drain with water
2. Dipose of kit waste in biohazard trash.
3. Wipe up spills with paper towels. Dipose in biohazard trash. Disinfect area with 70% EtOH.

5. What to do in case of emergency.

1. Yell for help.
2. Call 6-6002 or 911 after hours in case of emergency.
3. Qiagen contact: 1-800-362-7737 or www.qiagen.com