



LYSING MICRO-ORGANISMS WITH THE VK05 15 ML PRECELLYS® LYSING KIT

Bertin Technologies

/ CONTEXT

Microbes such as yeast or bacteria are difficult to lyse due to their thick cell wall, which makes nucleic acid extraction difficult. Gram positive bacteria for example, have a cell envelope comprised of the plasma membrane and a thick cell wall containing peptidoglycans. The most common technique for microbial extraction is the use of chemical lysis reagents such as ionic detergent followed by physical grinding with mortar and pestle in liquid nitrogen. The Precellys® homogenizers offer an alternative and more effective lysing solution for DNA and RNA extraction to be used for molecular biology research, such as sequencing, PCR or cloning.

With the new 15 ml tube designed for micro-organisms lysing, you can reach a higher volume (up to 9 ml) of lysed suspension at once.

/ MATERIALS

- Precellys® Evolution EQ02520-300-RD000.0
- Precellys® kit: 15ml VK05 tube (glass beads of 0,5 mm diameter) KT03961-1-504.15
- Samples: 9 ml of *E. coli* diluted 1/1000 ($3,15 \times 10^8$ CFU/ml)
- Others: PBS, Ringer and agar plate

/ PROTOCOL

Precellys® Evolution: 8500 rpm, 3x30 sec, 30 sec break

Culture:

- 3 dilutions : 1/1 - 1/10 - 1/100
- 100 µL / plate in duplicate
- 24 H - 37°C

Lysis in duplicate

Determination of the concentration:

- % mortality : $[(C_i - C_{grinding})/C_i] \times 100$

/ RESULTS

After 24h of incubation at 37°C, results were observed. The plate count is linear for *E. coli* over the range of 30 - 300 CFU on a standard sized Petri dish. We kept the two dilutions in this range to calculate the lysing efficiency.

CFU/plate	1/10 ²	1/10 ³	Concentration (CFU/mL)	% mortality
1/VK05_15mL	126/130	19/13	$1,3 \times 10^4$	96%
2/VK05_15mL	156/150	13/19	$1,5 \times 10^4$	95%

Table1: results of the lysing step with 9 ml of bacterial suspension, in duplicate. Each lysing suspension was spread on an agar plate in duplicate (100 µl).

Concentration of the starting solution was validated by spreading 100 µl on agar plate of corresponding dilution.



Figure1: E-coli culture

The VK05 15ml kit is very efficient as we observed percentage of mortality > 95% after lysing in comparison with the initial suspension of bacteria.

/ CONCLUSION

The Precellys® Evolution combined with the Precellys® lysing kit VK05 15 ml offers a quick and very efficient solution for preparation of micro-organisms suspension in large volumes. In addition to the capability of processing 6 tubes at once, equivalent to a midi prep, the preparation time is drastically decreased. The lysing efficiency is significantly increased.

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