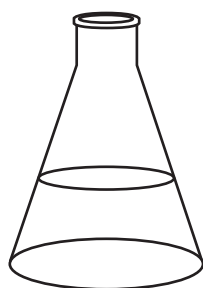


Plasmid DNA Purification Using NucleoBond Xtra Midi EF Kit



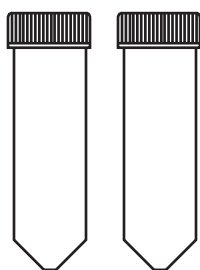
100 ml LB medium containing the appropriate antibiotic.

500 ml flask



Pick a single colony and inoculate LB medium.

Grow at 37°C for 12-16 h with shaking (approx. 220 rpm).



Transfer the culture to two 50-ml tubes.



Centrifuge at 4,500-6,000 x g (JA-14 rotor, 6,835-7,893 rpm) for 15 min at 4°C.

Resuspend the pellet in 8 ml of RES-EF (4 ml/50-ml tube) by pipeting.

Put all resuspension into a 50-ml tube.

Add 8 ml of LYS-EF, mix gently by inverting the tube 5 times.

Incubate the mixture at room temperature for 5 min.

Add 8 ml of NEU-EF.

Mix immediately and thoroughly by inverting 10-15 times.

Incubate crude **on ice** for 5 min.

Invert the tube 3 times and load lysate on NucleoBond Xtra Column Filter.



Equilibrate NucleoBond Xtra Column together with the inserted column filter with 15 ml of Equilibration Buffer EQU-EF.



Wash the filter with 5 ml of Buffer FIL-EF.



Discard the filter.

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Wash the column with 35 ml of ENDO-EF.

Wash the column with 15 ml of WASH-EF.

Elute the plasmid DNA with 5 ml of ELU-EF.



Collect the eluate into a 50 ml tube.

Add 3.5 ml (0.7 volumes) room temperature isopropanol to the eluted DNA.

Vortex well and let the mixture sit for 2 minutes.

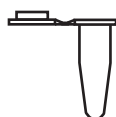
Load precipitation mixture into a NucleoBond Finalizer using 30-ml syringe.

Wash DNA pellet with 2 ml of room-temperature 70% EtOH.

Press air through the NucleoBond Finalizer (> 3 air).

Attach a 1-ml syringe to the NucleoBond Finalizer.

Elute DNA with 200-800 μ l H₂O-EF (usually 500 μ l).



Store at -20°C.

(Yield > 200 μ g)