

MaxiPlas™ maxiprep

from 10-500ml Bacterial Cell Suspension

For steps in this box only, in a 50ml centrifuge tube, **accumulate** desired cell pellet weight and then **use** the appropriate volume of #1 Buffer, #2 SDS, and #3 KAC, shown in table below.

For cell pellets weighing:

0.1 - 0.5 grams **use** 3.0ml of each reagent

0.5 - 1.0 grams **use** 4.0ml of each reagent

1.0 - 2.0 grams **use** 6.0ml of each reagent

2.0 - 3.0 grams **use** 8.0ml of each reagent

Resuspend cell pellet in #1 Buffer.

Add #2 SDS. Mix gently by inversion.
Incubate for 10 minutes @ RT.

Add #3 KAC. Mix gently by inversion.
Centrifuge for 10 minutes @ approx. 14,000xg.

Transfer supernatant to a new 50ml tube.
Carefully avoid precipitate. Discard pellet.

Add an equal volume of RT Isopropanol to supernatant. Centrifuge for 10 minutes.

Discard supernatant.

Resuspend pellet in 500µl of #1 Buffer.
Incubate for 10 minutes @ RT.

Transfer to a new 2.0ml ClickSeal micro tube.
Add 250µl of #2 SDS. Mix gently.
Add 250µl of #3 KAC. Mix gently.

Centrifuge for 10 minutes.

Transfer supernatant to a new 2.0ml micro tube.
Carefully avoid precipitate. Discard pellet.

Add an equal volume of RT Isopropanol to supernatant. Centrifuge for 10 minutes.

Remove supernatant completely. Dry pellet.

Depending on degree of purity desired, **either**:
(1) continue to next box, **or** (2) resuspend DNA in 10-100µl of H₂O and stop, **or** (3) resuspend DNA 10-100µl of H₂O and skip to GlasPac purification described in second box on page 6.

To further remove residual full-length RNA and chromosomal DNA, or when purifying larger 2 to 3 gram pellets, use optional lysis below:

Resuspend pellet in 300µl of #1 buffer.
Incubate for 10 minutes @ RT.

Add 100µl of #2 SDS. Mix gently.
Add 100µl of #3 KAC. Mix gently.
Centrifuge for 10 minutes.

Transfer supernatant to a new 2.0ml micro tube.
Add an equal volume of RT Isopropanol.
Centrifuge for 10 minutes.

Remove supernatant completely. Dry pellet.
Resuspend DNA in 10µl to 100µl of H₂O.

To remove residual proteins, oligo RNA, and inhibiting enzymes, continue with GlasPac purification below:

Add 500µl of #4 Salt to resuspended DNA.

Add 3µl of completely suspended GlasPac for the first µg of plasmid DNA, **plus** 1µl of GlasPac for each additional µg of plasmid DNA.

Vortex for 1 to 2 sec. Incubate for 5 minutes @ RT while periodically resuspending GlasPac.

Centrifuge for 10 seconds.

Aspirate supernatant with a MicroFlex pipet tip.

Resuspend GlasPac pellet in 500µl of #5 Wash.

Centrifuge for 10 seconds.

Aspirate supernatant with a MicroFlex pipet tip.

Repeat wash step with #5 reagent several times.

Aspirate supernatant efficiently with a MicroFlex pipet tip. Dry pellet.
(A quick spin helps to remove residual wash.)

Resuspend GlasPac pellet in an appropriate volume of H₂O (at least equal to the volume of GlasPac initially used).

Incubate for 5 minutes @ RT while periodically resuspending GlasPac.

Centrifuge for 30 seconds. Then transfer the eluted plasmid DNA to a new micro tube.